Opioid replacement therapy with methadone or buprenorphine effects on male mice reproduction

Fatemeh Moinaddini1 · Maryam Amirinejad1 · Tahereh Haghpanah1 · Mohsen Abedini1 · Farhad Yoosefi1 · Seyed Noureddin Nematollahi-mahani2,3

Received: 26 July 2022 / Accepted: 1 November 2022 / Published online: 17 November 2022
© The Author(s), under exclusive licence to Springer-Verlag GmbH Germany, part of Springer Nature 2022

Abstract

Rationale Opioid use disorders are commonly treated by long-acting agonist opioids including methadone and buprenorphine which could affect various aspects of male reproduction especially spermatogenesis.

Objectives We aimed to determine whether detoxification with methadone or buprenorphine was associated with reproductive disorders in male mice.

Methods We orally induced morphine dependence in NMRI male mice, and then performed detoxification programs using either methadone or buprenorphine. Testis architecture and sperm parameters including sperm nuclear DNA integrity, mitochondrial activity, oxidative stress in seminal plasma, and routine sperm parameters were assessed to find the involved mechanisms.

Results The number of Leydig cells and the thickness of germinal epithelium reduced following morphine use and increased differently after detoxification with methadone or buprenorphine. Morphine dependence and detoxification with methadone and buprenorphine had different effects on sperm parameters. Morphine altered chromatin integrity, mitochondrial activity, and oxidative stress in sperm. Detoxification with methadone improved mitochondrial activity but worsened chromatin integrity, whereas detoxification with buprenorphine improved neither chromatin integrity nor mitochondrial activity. Seminal plasma oxidative stress was higher in the treated groups compared to control groups but was comparable among treatment groups. Our study revealed that long-term morphine use followed by detoxification with methadone or buprenorphine impairs testis structure and sperm parameters. Detoxification from morphine use with methadone and buprenorphine led to different preclinical outcomes in semen quality parameters, including chromatin integrity. Therefore, clinical detoxification protocols should be performed more cautiously, considering the desire of the individuals to reproduce.

Keywords Morphine · Methadone · Buprenorphine · Detoxification · Testis · Sperm parameters

Significance statement Morphine-dependence alters sperm DNA chromatin and mitochondrial activity. Methadone detoxification improves mitochondrial activity but worsens chromatin integrity, while buprenorphine detoxification neither improves sperm DNA chromatin quality nor mitochondrial activity. It can be concluded that sperm parameters are differently affected by morphine, methadone, and buprenorphine use.

Introduction

Opium use is a long-lasting, worldwide habit in many societies including Iran which has an extended history of opium use, especially following endemics such as SARS-CoV-2 virus (Khosravi 2022). It has valuable narcotic and analgesic benefits, but long-term use of opium results in social and cognitional impairments also affects different body organs including the reproductive system (Ahmadnia et al. 2016a, b). Spermatozoa and all living cells live in aerobic condition and require oxygen. Oxygen metabolism generates reactive oxygen species (ROS), the increase of which can disrupt the cell membrane and increase DNA segmentation (Abdel-Zaher et al. 2013; Bakar et al. 2015), a phenomenon that is enhanced by morphine use via lipid peroxidation. In addition...
to excessive ROS formation following chronic morphine use, opioids may directly decrease testosterone production, affect semen quality, reduce sperm number, induce morphological changes in spermatozoa, and increase prolactin release (Bolelli et al. 1979).

Morphine, the most potent opium alkaloid, was first harvested by Sertturner in 1803, and its medicinal use quickly spread throughout the world due to its powerful analgesic properties (Takzare et al. 2016). Despite its potential to reduce pain, its chronic use actually leads to morphine dependence through its receptors scattered throughout the brain including the amygdala nucleus, hippocampus, thalamus, and hypothalamus (Vicente-Carrillo et al. 2016). The presence of opioid receptors in the testis can affect hypothalamic-pituitary gonadal axis and lead to dysfunction of the reproductive system, especially via direct and indirect effects of morphine on testis structure and function including testosterone. The failure of Leydig cells to produce testosterone (Jin and Yang 2014) affects sperm production, semen quality, sperm number, motility, and morphology (Safarinejad et al. 2013). As a solution to opium dependence, the use of opium substitutes including methadone and buprenorphine has been suggested over the years as opium replacement therapy protocols to alleviate signs and symptoms of morphine dependence (Dematteis et al. 2017).

Methadone, a long-acting opioid agonist, has been used for many years as the major alternative drug for detoxification in morphine-dependent individuals, reducing illicit drug use and improving rehabilitation (O’Connor 2005, Van den Brink and Haasen 2006). However, long-term use of methadone could result in muscle weakness, osteoporosis, testosterone deficiency, and infertility in human and animal models (Garrido and Trocóniz 1999, Trescot et al. 2008). Since 2001, buprenorphine, a semi-synthetic opioid that acts as a partial agonist at brain opioid receptor sites by blocking the action of full opioid agonists, has been extensively used for detoxification (Giacomuzzi et al. 2005). Buprenorphine is a favorable option for pregnant opioid-dependent women because it does not cross the placenta to affect the fetus directly (Robin et al. 2021). In addition, patients treated with buprenorphine had better erectile function compared with methadone replacement therapy (Bliesener et al. 2005).

There are some reports in the literature of changes in the reproductive system following morphine dependence in human and few studies in animal models. Opioid replacement therapy protocols which use methadone/buprenorphine reduce morphine dependence but may affect the reproductive system, the mode of action of which is not well studied (Shulman et al. 2021). To elucidate the mechanisms through which methadone/buprenorphine replacement therapy may affect testis structure, sperm quality, and quantity, as well as sperm fine structure, we first induced chronic morphine dependence in animals using oral morphine and then carried out a detoxification protocol using methadone and buprenorphine to evaluate testis structure by histological assessment of testsis, sperm quality, and quantity by routine analysis of sperm parameters, Sperm Chromatin DNA Dispersion Test (SCDT), sperm mitochondrial activity assessment, and measurement of the oxidative level of seminal plasma.

Materials and methods

Animals

The ethics committee at Kerman University of Medical Sciences, Kerman, Iran, approved the study (approval #: IR-KMU-REC-1397–361) in which 75 8-week-old male NMRI mice, with an average weight of 27 g, were purchased from the animal farm of Afzalipour Medical School, Kerman, Iran. Nine male mice were used for a pilot study and the remaining for the main work. The animals were kept in a 12/12-h day/night cycle, 25 ± 2 °C temperature, and free access to tap water and rodent food. Sixty-six male mice were divided into 6 groups of 11: (1) control group (40-day maintenance without any intervention, as age-matched group, Ctrl40), (2) morphine-dependent group (This group of mice received 0.4 mg/ml morphine in drinking water for 40 days, mrph40), (3) another control group (80-day maintenance without any intervention, as age-matched group, Ctrl80), (4) another morphine-dependent group (This group of mice received 0.4 mg/ml morphine in drinking water for 80 days, mrph80), (5) methadone group (see below for methadone detoxification protocol, Mtdn), and (6) buprenorphine group (see below for buprenorphine detoxification protocol, Bprn).

Experimental design

We dissolved 0.1 mg/ml morphine in the drinking water of mice and gradually increased it to 0.4 mg/ml in 15 days to induce morphine dependence in mice (Lewter et al. 2020). Three morphine-dependent mice were used to confirm morphine dependence by injection of 2 mg/kg naloxone and then assessing withdrawal symptoms. Since these mice showed withdrawal symptoms such as defecating and urinating, diarrhea, jumping, and wet dog shakes, the dependence of other mice was attributed to these mice (Moinaddini et al. 2021). Animals in the Ctrl40 and Mrph40 groups served as controls for animals in the Ctrl80 and Mrph80 groups, respectively, to nullify age-related changes in these groups. Animals in the Mtdn group were induced with morphine dependence (see above) for 40 days and then underwent methadone replacement therapy as described elsewhere (Hassan et al. 2020). Briefly, animals received 0.05 mg/day/i.p. methadone
(Tolid daru, Tehran, Iran) in normal saline followed by 0.135 mg/day/i.p. for 5 consecutive days. Twenty percent of the prescribed dose was reduced every 5 days until the end of the detoxification period (40 days). Animals in the Bprn group were induced with morphine dependence (see above) for 40 days and then underwent buprenorphine replacement therapy as described elsewhere (Bakhti-Suroosh et al. 2021). Briefly, animals received a subcutaneous injection of 0.018 mg buprenorphine in normal saline and then 0.036 mg/day for the next 4 days. Twenty percent of the prescribed dose was reduced every 5 days until the end of the detoxification period (40 days).

**Gross and morphometric examination of the testis**

The right testis was removed after deep anesthesia by ketamine and xylazine. Weight and the diameters were measured by a sensitive electrical balance and a digital caliper, respectively. The testis was then fixed in 10% formaldehyde (Dr. Mojalali Industrial Chemical Complex Co, Iran) in PBS for histologic evaluations.

**Histological studies**

A routine histologic processing was carried out on the samples including embedding in paraffin after dehydration and clearing. Five-micrometer-thick sections were prepared by a rotary microtome and stained by hematoxylin and eosin.

**Leydig cell count**

Leydig cells were counted in three interstitial spaces randomly selected from each section of the testicular tissue (eight sections; a total of 24 interstitial spaces), and the total number in each sample was reported.

**Thickness of the germinal epithelium and surface area of seminiferous tubule evaluation**

A total of 40 circular seminiferous tubules were randomly selected from each sample under an Olympus microscope equipped with a digital camera (×200). The germinal epithelium thickness (µm) was measured at 4 distinct points with the help of Hystolyb software (Zist rad, Tehran, Iran). Seminiferous tubule surface area was also measured with the same software, but it was reported in µm².

**Evaluation of the sperm parameters**

The mice were deeply anesthetized, the abdominal wall was opened, the cauda epididymis and the right vas deferens were carefully separated and placed in 1-ml pre-warmed HTF medium, and the samples were cut into several pieces and placed in a 37 °C humidified incubator with 5% CO₂ for 30 min. The tissue fragments were removed, and the sperm suspension was used for further investigations.

**Evaluation of the sperm number, viability, and motility**

Sperm parameter evaluation methods are described elsewhere (Basiri et al. 2011). Two examiners were trained, and the average of the observations by two examiners was recorded.

**Sperm motility**

To monitor the sperm motility, 10 µl of the sperm suspension was placed on a pre-warmed clean slide, covered with a coverslip, and evaluated under a light microscope (×400, Olympus, Japan). The sperms were classified into motile and non-motile.

**Sperm viability**

Eosin-Nigrosine (Merck, Germany) staining was used to determine sperm viability. Sperm with an intact membrane remain colorless (live) while sperm with damaged membrane stain pink (dead), due to eosin dye entering the cytoplasm. 5 µl of the sperm suspension was mixed with 5 µl of Eosin-Nigrosine dye, and a smear was prepared within 2 min. The sperm were examined by ×400 magnification of a light microscope.

**Sperm number**

To evaluate sperm number, a small amount of sperm suspension was mixed with 10% formaldehyde in phosphate-buffered saline and 50 g/L sodium bicarbonate. The suspension was transferred onto an improved Neubauer chamber, and the sperm were counted at four corners of the central square.

**Sperm morphology**

Sperm morphology was evaluated by analyzing the smears prepared from the right cauda epididymis by modified Papanicolaou staining method. A total of 200 spermatozoa per slide were examined to detect any abnormality in sperm head and tail.

**Evaluation of mitochondrial activity**

We used DAB (1 mg/ml in PBS) solution to evaluate the mitochondrial activity of sperms. Three parts of the sperm suspension were mixed with one part of DAB solution and incubated for 1 h at 37 °C in the dark, and then the smears
were prepared. The smear was fixed in 10% formaldehyde in PBS for 10 min, and after washing in tap water, the slides were air-dried and examined under a light microscope with ×1000 magnification. Depending on the proportion of the DAB deposition in the middle segment of sperm, mitochondrial activity was classified into class I: the middle segment is completely stained, class II: more than 50% of the middle segment is stained, class III: less than 50% of the middle segment is stained, and class IV: the middle segment is not stained at all (Fig. 1A).

**SCDT**

Sperm DNA Fragmentation (SDF) Assay Kit (Ibn Sina Center, Tehran, Iran) was used to evaluate sperm DNA fragmentation as follows: freshly prepared sperm samples were washed and diluted with PBS solution to obtain 5–10 million/ml sperm. Thirty microliters of sperm suspension was mixed with 70 μg agarose, spread on a clean glass slide previously coated with 0.65% normal agarose, and kept at 4 °C for 5 min to solidify the agarose gel. The slide was incubated in an acidic denaturing solution in the dark for 7 min, followed by incubating in a lubricating solution for 23 min; washing twice in PBS and dehydrating in the increasing concentrations of ethanol; staining by C, D, E solutions for 75, 120, and 60 s, respectively; and finally washing and drying in the air. The slide was observed under a light microscope (×1000) equipped with a digital camera. A picture was shot and using the Histolab software, the size of the halo around the sperm head was obtained and classified as follows (Fig. 1B): DNA defective sperm including sperm without halo and sperm with small halo, and healthy sperms without DNA defect including sperm with medium halo or sperm with a large halo (Safarinejad et al. 2013).

**Seminal plasma oxidative stress measurement**

Oxidative level of the seminal plasma was evaluated by malondialdehyde (MDA), the final product of polyunsaturated fatty acid peroxidation in the sperm. A stock solution of 15% w/v trichloroacetic acid in 0.25 N HCl and 0.375 w/v thiobarbituric acid in 0.25 N HCl was prepared. One volume of seminal plasma was mixed with two volumes of stock solution in a centrifuge glass tube and placed in a boiling water bath for 15 min. After cooling, the mixture was centrifuged at 1500 g for 10 min. The absorption of supernatant was measured at 532 nm wavelength by a spectrophotometer, and the MDA value in μM/ml was recorded against a standard curve.

**Statistical analysis**

We used a Windows compatible version of SPSS software (version 16) for data analysis. The normality of the data was analyzed by Kolmogorov–Smirnov test. We used one-way analysis of variance (ANOVA) followed by Tukey’s post hoc test for multiple comparisons on the mean of different variables. The data were presented as mean ± SEM. *P < 0.05* was considered statistically significant.

**Results**

**Weight and dimensions of testis**

The weight of testes decreased significantly (*P < 0.05*) in Mrph40 and Mtdn groups compared to Ctrl40 and Ctrl80 groups. The length of the testes differed significantly (*P < 0.05*) in the mice treated with methadone compared to Ctrl40 and Ctrl80 groups. The width of the testes differed...
significantly \((P < 0.05)\) in Mrph80 and Mtdn groups compared to Ctrl40 and Ctrl80 groups and also that of Mrph40 group (Table 1).

**Histological evaluations**

**Leydig cell number**

Induction of morphine dependence for 40 days (Mrph40 group) significantly \((P < 0.001)\) reduced the number of Leydig cells \((171.87 \pm 7)\) compared to Ctrl40 \((226.75 \pm 9.06)\) group. This reduction continued when the morphine dependence lasted for 80 days \((171.87 \pm 7.1\) and \(135.12 \pm 4.36\) for the Mrph40 and Mrph80, respectively). Interestingly, detoxification with methadone \((141.62 \pm 3.43)\) and especially buprenorphine \((151.5 \pm 3.56)\) significantly \((P < 0.01, \text{compared to Mrph80 group, } 135.12 \pm 4.36)\) increased the number of Leydig cells. However, none of the treated groups (Mrph80, Mtdn, and Bprn) could reach the value of their counterpart (Ctrl80) group (Table 1).

**Germinal epithelium**

Increasing the age of the animals did not significantly change the thickness of the germinal epithelium \((96.04 \pm 1.71\) and \(91.5 \pm 1.69\) for Ctrl40 and Ctrl80 groups, respectively). While, after 40 days, dependence on morphine germinal epithelium thickness \((72.56 \pm 1.33)\) significantly \((P < 0.001\) compared with Ctrl40) decreased, also after 80 days, dependence on morphine \((70.5 \pm 1.24, P < 0.001\) compared with Ctrl80). Detoxification with methadone \((78.65 \pm 2.39)\) and buprenorphine \((86.43 \pm 2.3)\) significantly increased the epithelium thickness compared with the Mrph80 \((70.5 \pm 1.24)\) group \((P < 0.01\) and \(P < 0.001\) compared with the Mtdn and Bprn groups, respectively). When the value was compared between Mtdn and Bprn groups, a significant level \((P < 0.05)\) was also observed (Table 1).

**Seminiferous tubule surface area**

Seminiferous tubular area did not significantly change with age in Ctrl40 compared to the value in Ctrl80. Dependence of animals on morphine for 40 and 80 days \((37,766.1 \pm 12\) and \(36,625.1 \pm 6.6, \text{respectively})\) significantly decreased the tubular surface area compared to the control groups \((P < 0.05; \text{Mrph40 compared to Ctrl40, } 46,216.2 \pm 19.4, \text{and} \ P < 0.01; \text{Mrph80 compared to Ctrl80, } 46,926.2 \pm 23)\). The lowest tubular surface area was detected in the Mtdn group \((15,423.6 \pm 1972.2)\) and the highest \((47,436.1 \pm 2667.7)\), close to the untreated control groups (Ctrl40 and Ctrl80), in the Bprn group (Table 1).
Sperm parameters

Sperm number

The values among different groups were not statistically significant. However, treatment of animals for 80 days with morphine reduced sperm number \(8.8 \times 10^6 \pm 2.32\), while detoxification of animals with methadone and buprenorphine increased sperm number \(18.4 \times 10^6 \pm 3.73\) and \(17.2 \times 10^6 \pm 3.67\), respectively, to a higher level than the Ctrl80 group \(14 \times 10^6 \pm 2.12\) (Fig. 2A).

Sperm viability

Sperm viability was comparable between the Ctrl40 \((68.5 \pm 2.19)\) and Ctrl80 \((62.37 \pm 3.11)\) groups but decreased in all treated groups. The highest decrease was detected in Mrph80 group \((58.8 \pm 2.96)\), followed by Bprn \((59.4 \pm 7.29)\) and Mrph40 \((62.37 \pm 3.11)\). A significant \((P < 0.01)\) reduction in sperm viability was observed in the Mrph80 group \((58.78 \pm 2.96)\) compared to the Ctrl80 group. It is interesting to note that in methadone detoxified animals \((66.64 \pm 2.9)\) compared to the Mrph80 group \((58.8 \pm 2.96)\), sperm viability significantly increased \((P < 0.05)\), (Fig. 2B).

Sperm motility

Sperm motility assessments in the studied groups showed a significant decrease in all the treated groups compared to the control groups (Fig. 2C). However, increasing age did not significantly reduce sperm motility neither in the control groups nor in the morphine-dependent groups. Dependence on morphine \((43.4 \pm 2.8)\) and detoxifying with methadone \((45.37 \pm 2.31)\) and buprenorphine \((38.42 \pm 4.82)\) significantly \((P < 0.01\) and \(P < 0.001\), respectively) decreased sperm motility compared to their values with the control groups (Ctrl40 and Ctrl80, \(67.57 \pm 2.7\) and \(62.7 \pm 2.32\) respectively).

Sperm normal morphology

Sperm normal morphology did not change considerably over time. It was comparable between Ctrl40 \((91.75 \pm 0.8)\) and Ctrl80 \((90.22 \pm 1.37)\) groups. However, a significant \((P < 0.001)\) decrease was observed in the morphine-treated (Mrph40 and Mrph80, \(79 \pm 2.59\) and \(79.11 \pm 1.65\), respectively) groups compared to the controls (Ctrl40 and Ctrl80, respectively). Interestingly, detoxification with buprenorphine \((84.89 \pm 2.17)\) increased sperm normal morphology to a higher value than mrph80 group, but not than Ctrl80 group (Fig. 2D). Although treatment of animals with methadone

Fig. 2. Sperm number (A), viability (B), motility (C), and normal morphology (D) in different groups. Data are presented as mean±SEM of at least 10 samples. Compared with a control group (40-day), b morphine group (40-day), c control group (80-day), d morphine group (80-day), e methadone group. * and ** significantly different at \(P < 0.05\) and \(P < 0.01\), respectively, analyzed by one-way ANOVA followed by Tukey post hoc test.
(79.91 ± 1.76) increased the normal morphology value non-significantly compared to the Mrph80 group, it was still significantly (P < 0.01) lower than the value in Ctrl80 group.

**Sperm mitochondrial activity**

Sperm mitochondrial activity was assessed following the reaction of sperm mitochondria in the mid-piece with DAB solution. The brown color of the mid-piece was graded as class I to IV (class I being the most active and class IV being the least active). For ease of analysis, class I + II values and class III + IV values were combined. Mitochondrial activity (class I + II) in Mrph80 (43.4 ± 5.49), Mtdn (62.2 ± 32.98), and Bprn (42.39 ± 6) groups was significantly lower than in Ctrl80 (86.1 ± 28.71) groups. In addition, the mitochondrial activity of the Mrph40 group (64.01 ± 3.0) was significantly (P = 0.015) lower than that of the Ctrl40 group (92.39 ± 10). Treatment of animals for 80 days with morphine significantly (P ≤ 0.001) decreased mitochondrial activity compared to the Ctrl80 group. Detoxification with methadone significantly (P < 0.05 compared to Mrph80) increased the value, but detoxification with buprenorphine did not change the outcome when comparing the values with Mrph80 group (Table 2).

**Sperm chromatin DNA dispersion test**

The halo diameter around the sperm head was assessed in an agarose gel–based test to determine sperm nuclear DNA fragmentation (Table 2). Aging non-significantly increased SDF (Ctrl40, 3.05 ± 0.68 compared with Ctrl80, 10.71 ± 1.8). However, treatment of animals with morphine for 40 days had little effect on SDF (Mrph40, 4.99 ± 1.6 compared with Ctrl40, 3.05 ± 0.68), but treatment of animals for 80 days with morphine significantly (P < 0.01) increased SDF (Mrph80, 20.59 ± 4.5) compared with Ctrl80, 10.71 ± 1.8). The highest level of SDF was observed in the methadone-treated group (27.29 ± 3.7). It is noteworthy that neither methadone nor buprenorphine detoxification significantly decreased SDF level (Mtdn, 27.29 ± 3.7 and Bprn, 22.4 ± 2.7) compared to the Mrph80 group (20.59 ± 4.5).

**Seminal plasma oxidative stress**

Seminal plasma oxidative stress evaluated by the MDA measurement was comparable between Ctrl40 (4.0 ± 0.12) and Ctrl80 (4.0 ± 0.44) groups. The amount of MDA was high in the Mrph40 (5.0 ± 0.59) group and higher in Mrph80 (6.0 ± 0.47) as well as each of the two detoxified (Mtdn, 6.0 ± 037 and Bprn, 6.0 ± 0.29) groups (Fig. 3). However, statistical analysis did not detect significant differences between groups.

**Discussion**

In the present study, sperm and testis parameters were evaluated in the morphine-dependent animals and also in the detoxified animals which received methadone/buprenorphine for 40 days. The effects of morphine on the germ cells and the glandular cells population of reproductive system

---

**Table 2** The sperm mitochondrial activity and sperm DNA dispersion test (SCDT) values in different groups of animals

| Parameters | Ctrl40 | Mrph40 | Ctrl80 | Mrph80 | Mtdn | Bprn |
|------------|--------|--------|--------|--------|------|------|
| Class I + class II | 92.39 ± 10.0 | 64.01 ± 3.0a** | 86.1 ± 28.71 | 43.4 ± 5.49ac*** | 62.2 ± 32.98ac**d* | 42.39 ± 6abc***b*c** |
| Class III + class IV | 8.39 ± 1.0 | 36 ± 3.01a** | 13.1 ± 1.71 | 56.4 ± 5.49ac*** | 37.2 ± 17.9ac**d* | 57.59 ± 6.39abc***b*c** |
| High SDF | 3.05 ± 0.68 | 4.99 ± 1.6 | 10.71 ± 1.8 | 20.59 ± 4.5a**b** | 27.29 ± 3.74ab**c** | 22.4 ± 2.7ab*** |
| Low SDF | 96.94 ± 0.68 | 95 ± 1.56 | 89.28 ± 1.7 | 79.39 ± 4.4a**b** | 73.41 ± 3.8ab**c** | 77.94 ± 2.76a**b** |

Data are presented as mean ± SEM of at least 10 animals per group. a: control group (40-day), b: morphine group (40-day), c: control group (80-day), d: morphine group (80-day), e: methadone group. Data were analyzed by one-way ANOVA followed by Tukey post hoc test.

*Significantly different at P < 0.05; **P < 0.01; ***P < 0.001

---

![Fig. 3](image-url)
have been investigated to some extent. It appears that morphine and its families can directly act on the opioid receptors present in the testis (Subirán et al. 2011). In the living cells, three mechanisms are involved in the DNA fragmentation: apoptosis, abnormal chromatin packaging, and oxidative stress. In mammalian testis, germ cells proliferate several times before the onset of meiosis, which results in the spermatozoa production. In the testis, apoptosis is responsible for population control and selection of germ cells (Shafik et al. 2006). In some cases, however, testis initiates germ cell apoptosis, but several cells may escape this process and continue to mature, leading to poor sperm quality with apopotic traits such as external phosphatidylserine and DNA fragmentation (Amin 2013; Ahmadnia et al. 2016a, b).

Drug use in humans significantly increases DNA fragmentation, and a dose-dependent relationship between drug use, sperm DNA fragmentation, and degradation of their sperm parameters including sperm number has been suggested (Wright et al. 2014). It has been reported that high DNA fragmentation rate is caused by an imbalance between antioxidant capacity and ROS production in seminal plasma, which leads to sperm quality damage and male infertility (Safarinejad et al. 2013). In the present study, a higher DNA fragmentation rate was observed in Mrph80, Bprn, and also Mtdn groups than what was observed in their control (Ctrl80) group. Interestingly, although not significantly different, DNA fragmentation value in the morphine- and buprenorphine-treated animals was higher than 80-day morphine-treated animals (Mrph80 group), a finding that should be taken into consideration when implementing detoxification programs in humans. Oxidative stress level, measured by MDA level in seminal plasma, was not significantly different between groups. However, it was interestingly higher in the groups with higher DNA fragmentation rate (Mrph80, Mtdn, and Bprn). In addition to altered sperm DNA fragmentation in the morphine-dependent animals as well as detoxified animals, mitochondrial activity measured by DAB incorporation into sperm mitochondria in morphine-dependent groups (Mrph40 and Mrph80) and both detoxified groups (Mtdn and Bprn) was affected. It is interesting to note that the rate of class I + II sperms in the Mtdn group was significantly higher than that in the Mrph80 and Bprn groups, a finding that was confirmed by changing sperm motility in different groups; sperm motility in animals treated with morphine for 80 days or detoxified by methadone and/or buprenorphine was significantly lower than the sperm motility in the Ctrl80 group. The motility potential of sperm directly depends on the level of ATP production in the mitochondria. Two mechanisms have been proposed to explain reduced sperm motility: a reduction in mitochondrial energy production and any damage to axonal proteins (Aitken and Sawyer 2003; Turner 2003). Sperm motility, which is highly correlated with the male fertility, is reduced in opium users (Ragni et al. 1988). Morphine, a full agonist of µ receptors, reduces both the proportion of progressive motile sperm and sperm velocity without altering the pathway, which is reversed by the antagonist naloxone (Agirregoitia et al. 2012). Opium and heroin users have also experienced significantly lower sperm motility compared to control subjects (Assaei et al. 2013). Overexpression of ROS may cause mutations in the mitochondrial genome disrupting normal shape and function of the adult sperm (Jalili et al. 2014). Morphine-induced oxidative stress rapidly reduces ATP level. Consequently, it reduces sperm motility and viability (Pena et al. 2003). In a study conducted on three groups of subjects with varicocele and heavy or moderate smoking habits, class I mitochondrial activity and sperm progressive movement rate was significantly altered in heavy-smoker subjects (Fariello et al. 2012).

The results of the present study showed that sperm viability in the Mrph80 group significantly decreased compared with the Ctrl40, 80, and Mtdn groups. Detoxification of animals with buprenorphine significantly decreased sperm viability, while methadone detoxification increased sperm viability. On the other hand, detoxification with buprenorphine increased sperm normal morphology rate compared to Mrph80 and Mtdn groups. Kobayashi et al. (1991) showed that a decrease in the number of viable sperm is associated with an increase in the ROS level because it increases cellular membrane degradation following an increase in the MDA level. In addition to the deleterious effects of morphine on sperm parameters, morphine administration affects different cell types, especially highly sensitive testicular cells, through excess ROS production, which in turn results in an imbalance in testis weight and dimensions (Kobayashi et al. 1991). Ghowsi and Yousofvand (2015) reported significant weight loss in the testis, prostate, and seminal vesicles following dependence of adult rats to morphine (Ghowsi and Yousofvand 2015). Also, in the study of Lakhman et al. (1989), 5 to 10 days of methadone administration significantly reduced the weight of the sex organs in mice (Lakhman et al. 1989). In the report of Jalili et al., a reduction in the size of seminiferous tubules and the number of sperm along with an increase in the level of ROS and free radicals that lead to a decrease in the testis weight in morphine users was identified (Jalili et al. 2016). However, Bu et al. (2011) stated that testis atrophy which happens following morphine administration is mostly due to some unknown factors that interfere with spermatogenesis and thus reduces the number of germ cells (Bu et al. 2011). In our study, treatment of animals with morphine for 40 and 80 days as well as detoxification with methadone caused a significant decrease in testis weight. This was also true for seminiferous tubular surface area and germinal epithelium thickness, which were significantly reduced in morphine-dependent animals.
be chosen more carefully with regard to the propensity of morphine-dependent individuals to reproduce.

Author contribution Participated in research design: Nematollahi-mahani, Haghpanah, Moinaddini.
Conducted experiments: Moinaddini, Abedini, Yoosofi.
Performed data analysis: Moinaddini, Haghpanah.
Wrote or contributed to the writing of the manuscript: Amirinejad, Moinaddini, Haghpanah, Nematollahi-mahani.

Funding Kerman University of Medical Sciences Research affair provided us the financial support.

Data availability The datasets used and analyzed during the course of this study are available from the corresponding author upon reasonable request.

Conclusions
Conflict of interest The authors declare no competing interests.

References
Abdel-Zaher AO, Mostafa MG, Farghaly HS, Hamdy MM, Abdel-Hady RH (2013) Role of oxidative stress and inducible nitric oxide synthase in morphine-induced tolerance and dependence in mice. Effect of alpha-lipoic acid. Behav Brain Res 247:17–26
Agirregoitia E, Subiran N, Valdivia A, Gil J, Zubero J, Irazusta J (2012) Regulation of human sperm motility by opioid receptors. Andrology 44:578–585
Ahmadnia H, Akhavan Rezayat A, Hoseyni M, Shariﬁ N, Khajedalooee M, Akhavan Rezayat A (2016a) Short-period influence of chronic morphine exposure on serum levels of sexual hormones and spermatogenesis in rats. Nephrourol Mon 8(4):e38052
Ahmadnia H, Rezayat AA, Hoseyni M, Shariﬁ N, Khajedalooee M, Rezayat AA(2016b) Short-period influence of chronic morphine exposure on serum levels of sexual hormones and spermatogenesis in rats. Nephrourology monthly 8(4)
Aitken RJ, Sawyer D (2003) The human spermatozoon—not waving but drowning. Advances in male mediated developmental toxicity 85–98
Amin YK (2013) The relation of opium addiction and reproductive toxicity in male rats: a histological and hormonal study. Zanco J Med Sci Zanco J Med Sci 17(1):311_316-311_316
Assaei R, Nazari H, Pajouhi N, Zahed-Asl S (2013) Pituitary-gonadal axis hormone and semen analysis in narcotic dependency. Zahedan Journal of Research in Medical Sciences 15(4)
Babaei H, Sepehri G, Kheirandish R, Abshenas J, Monshi M (2012) The effects of long-term administration of buprenorphine on blood testosterone level and morphometrical and histopathological changes of mouse testis. Comp Clin Pathol 21(6):1527–1532
Bakar NHA, Hashim SN, Mohamad N, Husain R, Adnan LHM, Shariff H, Zakaria NH (2015) Role of oxidative stress in opiate withdrawal and dependence: exploring the potential use of honey. J Appl Pharma Sci 5(12):159–161
Bakhti-Sarosh, A, Towers EB, Lynch WJ (2021) A buprenorphine-validated rat model of opioid use disorder optimized to study sex differences in vulnerability to relapse. Psychopharmacology 1–18
Basiri M, Abadipour ME, Djahromi VH, Zandi NS, Azad AS, Nematollahi-Mahani SN (2011) Effects of nicotine and ethanol administration on the seminal vesicle of adult rats. Journal of Reproduction & Infertility 12(2)

Conclusions

Our findings clearly show that chronic morphine use affects the reproductive system of mice, especially the number of Leydig cells and sperm parameters. Methadone detoxification decreases sperm chromatin quality but increases mitochondrial activity. In contrast, buprenorphine detoxification increases sperm chromatin quality but decreases sperm mitochondrial activity. Other sperm parameters and fine architecture of testis are also differently affected by methadone or buprenorphine detoxification. If the data on sperm parameters and fine structure are confirmed in human studies, morphine replacement therapy protocols in humans should
Bbiesner N, Albrecht S, Schwer A, Weckbecker K, Lichtermann D, Klingmuller D (2005) Plasma testosterone and sexual function in men receiving buprenorphine maintenance for opioid dependence. J Clin Endocrinol Metab 90(1):203–206

Bolega G, Lafiscia S, Flamigni C, Lodi S, Franceschetti F, Filicori M, Mosca R (1979) Heroin addiction: relationship between the plasma levels of testosterone, dihydrotestosterone, androstenedione, LH, FSH, and the plasma concentration of heroin. Toxicology 15(1):19–29

Bu T, Mi Y, Zeng W, Zhang C (2011) Protective effect of quercetin on cadmium-induced oxidative toxicity on germ cells in male mice. Anat Rec Adv Integ Anat Evol Biol 294(3):520–526

Dematteis M, Auricacome M, D’Agnone O, Somaini L, Szerman N, Littlewood R, Alam F, Alho H, Benyamina A, Bobes J (2017) Recommendations for buprenorphine and methadone therapy in opioid use disorder: a European consensus. Expert Opin Pharmacother 18(18):1987–1999

Fariello RM, Pariz JR, Spaine DM, Gozzo FC, Filicori M, Lafisca S, Flamigni C, Lodi S, Franceschetti F, Filicori M, Mosca R (1979) Heroin addiction: relationship between the plasma levels of testosterone, dihydrotestosterone, androstenedione, LH, FSH, and the plasma concentration of heroin. Toxicology 15(1):19–29

Ghowsi M, Yousofzand N (2015) Impact of morphine dependency and detoxification by methadone on male’s rat reproductive system. Iran J Reprod Med 13(5):275

Giacomuzzi SM, Ertl M, Kemmler G, Riemer Y, Vigl A (2005) Sublingual buprenorphine and methadone maintenance treatment: a three-year follow-up of quality of life assessment. Sci World J 5:542–548

Haddadi M, Ai J, Shirian S, Kadivar A, Farahmandfar M (2019) The effect of methadone, buprenorphine, and shift of methadone to buprenorphine on sperm parameters and antioxidant activity in a male rat model. Comp Clin Pathol 1–8

Hassan R, Pike See C, Sreenivasan S, Mansor SM, Muller CP, Hassan Z (2020) Mitragynine attenuates morphine withdrawal effects in rats—a comparison with methadone and buprenorphine. Front Psych 11:411

Jalili C, Salahshoor MR, Naseri A (2014) Protective effect of Urtica dioica L against nicotine-induced damage on sperm parameters, testosterone and testis tissue in mice. Iranian J Reprod Med 12(6):401

Jalili C, Ahmadi S, Roshankhah S, Salahshoor M (2016) Effect of Genistein on reproductive parameter and serum nitric oxide levels in morphine-treated mice. Int J Reprod Biomed 14(2):95

Jin J-M, Yang W-X (2014) Molecular regulation of hypotalamus–pituitary–gonad axis in males. Gene 551(1):15–25

Khosravi M (2015) Increasing opium use in Iran in response to unsubstantiated rumors that it protects against COVID-19. Addiction (abingdon, England) 117(4):1173

Kobayashi T, Miyazaki T, Natori M, Nozawa S (1991) Protective role of superoxide dismutase in human sperm motility: superoxide dismutase activity and lipid peroxide in human seminal plasma and spermatozoa. Hum Reprod 6(7):987–991

Lakhan SS, Singh R, Kaur G (1989) Morphine-induced inhibition of ovulation in normally cycling rats: neural site of action. Physiol Behav 46(3):467–471

Lewter LA, Johnson MC, Treat AC, Kassick AJ, Averick S, Kolber BJ (2020) Slow-sustained delivery of naloxone reduces typical naloxone-induced precipitated opioid withdrawal effects in male morphine-dependent mice. J Neurosci Res

Moinaddini F, Haghpanah T, Esfahlani MA, Amirinejad M, Nemattollahi-Mahani SN (2021) The effects of morphine dependence and detoxification with methadone and buprenorphine on sexual behavior and sex hormones. International Journal of High Risk Behaviors and Addiction 10(1)

O’Connor PG (2005) Methods of detoxification and their role in treating patients with opioid dependence. JAMA 294(8):961–963

Pena F, Johannisson A, Wallgren M, Martinez HR (2003) Antioxidant supplementation in vitro improves boar sperm motility and mitochondrion membrane potential after cryopreservation of different fractions of the ejaculate. Anim Reprod Sci 78(1–2):85–98

Raglin G, de Lauretis L, Bestetti O, Sghedoni D, ARO V G A (1988) Gonadal function in male heroin and methadone addicts. Int J Androl 11(2):93–100

Robin AM, Hersh AR, John C, Cagheay AB (2021) Cost effectiveness of buprenorphine vs. methadone for pregnant people with opioid use disorder. J Matern Fetal Neonatal Med 1–9

Safarinejad MR, Asgari SA, Farshi A, Gholi AA, Iravani S, Khoshdel AR (2013) The effects of opiate consumption on serum reproductive hormone levels, sperm parameters, seminal plasma antioxidant capacity and sperm DNA integrity. Reprod Toxicol 36:18–23

Shakif A, Shakif A, Sibai OE, Shakif I (2006) Sperm DNA Fragm Arch Androl 52(3):197–208

Shulman M, Cho T-H, Scodes J, Pavlicova M, Wai J, Haemlein P, Tofighi B, Campbell AN, Lee JD, Rotrosen J (2021) Association between methadone or buprenorphine use during medically supervised opioid withdrawal and extended-release injectable naltrexone induction failure. J Subst Abuse Treat 108292

Subirán N, Casis L, Irazusta J (2011) Regulation of male fertility by the opioid system. Mol Med 17(7):846–853

Takzare N, Samizadeh E, Shoar S, Zolbin MM, Naderan M, Lashkari A, Bakhtiarain A (2016) Impacts of morphine addiction on spermatogenesis in rats. Int J Reprod BioMed 14(5):303

Trescot AM, Helm S, Hansen H, Benyamin R, Glaser SE, Adlaka R, Patel S, Manchikanti L (2008) Opioids in the management of chronic non-cancer pain: an update of American Society of the Interventional Pain Physicians’ (ASIPP) guidelines. Pain Physician 11(2 Suppl):S5–S62

Turner RM (2003) Tales from the tail: what do we really know about sperm motility? Journal of Andrology 24(6):790–803

Van den Brink W, Haasen C (2006) Evidenced-based treatment of opioid dependence and detoxification by methadone on sexual function in males receiving buprenorphine maintenance for opioid dependence. JAMA 294(8):961–963

Vinicio-Carrillo A, Alvarez-Rodriguez M, Rodriguez-Martinez H (2016) The mu (μ) and delta (δ) opioid receptors modulate boar sperm motility. Mol Reprod Dev 83(8):724–734

Wright C, Milne S, Leeson H (2014) Sperm DNA damage caused by smoking and treatment in fertile men. Andrology 2014:55–61

Publisher’s note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.