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The effects of opiate consumption on serum reproductive hormone levels, sperm parameters, seminal plasma antioxidant capacity and sperm DNA integrity

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ABSTRACT

We evaluated the effects of opiate consumption on semen quality, sperm function, seminal plasma antioxidant capacity, and sperm DNA integrity. A total of 142 opiate addict men (group 1) were enrolled in the study and 146 healthy age matched male volunteers (group 2) served as controls. Two semen analyses were performed in all participants. Sperm chromatin structure assay (SCSA) was used to identify sperm DNA integrity. The mean ± SD sperm concentration in opiate users and in control subjects was 22.2 ± 4.4 and 66.3 ± 8.3 million per ml, respectively ($P = 0.002$). A significant increase in the amount of fragmented DNA was found in opiate consumers compared with that in controls (36.4 ± 3.8% vs. 27.1 ± 2.4%, $P = 0.004$). Significantly decreased levels of catalase-like and superoxide dismutase-like (SOD) activity were observed in group 1 compared with group 2. Opiate consumption has significant adverse effects on semen quality. In cases of unexplained infertility in men, opium consumption should be considered as a possible factor.

1. Introduction

Addiction to mind altering and recreational drugs is increasingly becoming a major worldwide medical and social problem that is prevalent in rich and poor countries similarly [1]. A considerable number of male factor infertility is categorized as "idiopathic" due to unknown etiology [2]. In clinical practice, we regularly encounter men with poor semen quality, who are taking opiate and/or recreational substances. Endogenous opioid peptides are present in various tissues of the male reproductive tract, suggesting that they may be involved in the reproductive function [3]. Several studies have demonstrated the deleterious effects of opiate compounds on sperm cell motility [4] and morphology [5]. It has been demonstrated that opiate abuse may result in hypogonadism, primarily by decreasing release of gonadotropin-releasing hormone (GnRH) [6]. In an extensive literature review, Vuong et al. demonstrated that sex hormone binding globulin is elevated and free and total testosterone are decreased in opiate consumers [6]. Opioid peptides exert their effects through δ-, κ-, and μ (DOR, KOR, and MOR, respectively) opioid receptors. Agirregoitia et al. reported for the first time the presence of these three opioid receptors in human spermatozoa membranes [7]. Besides these opioid receptors, leuenkephalin, met-enkephalin and β-endorphin immunoreactivity has also been recognized in spermatozoa [8]. The main sperm pathology in heroin (μ-agonist) addicts is decreased motility [9]. In addition, chronic heroin abuse has been extensively linked to oxidative stress [7]. Cicero et al. demonstrated that, chronic opiate exposure in the male rat adversely affects fertility [10].

Chronic cocaine administration to peri-pubertal rats results in spermatogonia with DNA displaying a distinct ladder pattern [11]. Fronczak et al. reviewed the literature on the prevalence and effects of illicit drug use on male fertility [12]. They concluded that illicit substances may negatively affect male fertility. Acute injection of methamphetamine induces apoptosis in seminiferous tubules in male mice testis and DNA ladders [13]. Chronic exposure to 3,4-methylenedioxy-N-methylamphetamine (MDMA, ecstasy) changes testes histopathology and increases DNA damage in sperm in male rats [14]. Oxidative stress is a significant factor in the

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etiology of male infertility and it can lead to an increased DNA fragmentation [15,16]. Imbalances between reactive oxygen species (ROS) production and semen antioxidant capacity will result in oxidative stress [17]. There is increasing evidence that one mechanism of ecstasy-induced toxicity is the production of reactive oxygen and reactive nitrogen species and a consequent production of oxidative/nitrosative stress [18]. Given the potential adverse effects of opiate on male fertility we aimed to determine the semen quality, serum reproductive hormones, seminal plasma antioxidant activity, and the rate of sperm DNA damage in opiate addict men.

2. Materials and methods

2.1. Study participants

In a case control study, 186 men aged 20–50 years were screened for eligibility (cases, group 1). They were enrolled from Addiction Treatment Centers before entering to treatment programs. Recruited men were required to meet Diagnostic and Statistical Manual of Mental Disorders, 4th edition (text revision) criteria for addiction [19]. Controls (group 2) consisted of 188 healthy age matched male volunteer blood donors without a history of problems with fertility. Participants were enrolled using local advertisements for a study of fertility capacity. No participants were on medications known to affect spermatogenesis. The study requirements were carefully explained to participants and all subjects provided informed consent before entering the study, which was conducted in accordance with the Declaration of Helsinki. The Medical Ethics Committee at the study site approved the study protocol. No remuneration was offered.

2.2. Inclusion/exclusion criteria

Participants were included in the study if they were between 20 and 50 years. None of the participant had known medical or surgical condition that could influence their fertility. Subjects with a history of any genitourinary surgery, azoospermia, epididymo-orchitis, varicocele, cryptorchidism, alcohol consumption, sexually transmitted disease, any toxin exposure or concomitant medical problems known to be associated with decreased fertility were excluded from study. Other exclusion criteria were any neurological or psychiatric disease, a history of any known endocrine abnormality, present use of ergogenic aids such as creatine monohydrate, or any anabolic medications such as steroids; Y chromosome microdeletions or karyotype abnormalities; and body mass index (BMI) $\geq$30. Polyconsumers were also excluded from the study. Of screened cases and controls 142 and 146 subjects met study criteria and were recruited into the study.

2.3. Evaluations

All participants underwent complete physical examination with special attention to genitourinary system. All men completed a detailed questionnaire, including demographic data, information on marriage, medical or surgical illness, drug use, chemical exposure, smoking history and other information that may affect fertility capacity. The gathered data regarding the substance-use included duration of dependence, main type of opioid used, treatments received, the route of administration (ingestion, intravenous injection, sniffing, smoking), and the amount consumed. Two main types of opiate which are used in Iran are Teriak (crude opiate) and Shireh (a refined opiate extract). The average amount of opium consumption per day is presented as "Nokhod", the local unit of opium use equivalent to 0.1935 g.

Laboratory tests included urine analysis, serum biochemistry, hematological tests, serum levels of luteinizing hormone (LH), follicle stimulating hormone (FSH), testosterone (T), free testosterone (fT), prolactin (PRL), sex hormone binding globulin (SHBG), free thyroxin (fT4), free triiodothyronin (fT3), total bilirubin, aspartate (AST) and alanine aminotransferase (ALT), alkaline phosphatase (ALP), creatinine, and electrolytes. Genetic testing included Karyotype analysis and Y chromosome microdeletion detection. In every subject the immunobead test for antisperm antibody binding and acrosome reaction assay as a sperm function test were done. Total testicular volume was measured by ultrasonography.

2.4. Semen analysis

Each participant collected a semen sample into a sterile container after 3–5 days of abstinence from ejaculation. All of the samples were provided on site. At least two semen samples were collected for analysis from each participant 4 weeks apart. When values differed by more than 20%, a third test was performed (n = 24, 8.3%). In these cases all 3 tests were evaluated. Samples were examined at least twice for volume, sperm concentration, progressive and total motility, and the percent of sperm with normal forms. The duplicate semen analysis of each sample was conducted by the same technicians. Each technician analyzed approximately the same number of samples and analyzed samples from both groups. Four technicians performed all semen analyses. Standardized semen analysis methods were taught to each technician by one of the authors (Safarinejad MR). Quality control and expertise criteria were set for less than 15% individual technician variability to confirm that technicians were fully trained to conduct the semen analysis. An additional quality control procedure included the blind examination of the same 15 slides at intervals of every other week by the technician. All semen analyses were performed in compliance with 1992 WHO criteria [20]. The normal WHO values included an ejaculate volume of more than 2.0 ml, sperm concentration of 20×10^6 spermatozoa/ml or greater, 30% or more with normal forms, and motility of 50% or more with forward progression. The total motile sperm count was calculated using the formula (semen volume $\times$ sperm concentration $\times$ motility percent)/100. An average of two semen analyses results for each subject and each sperm parameter was taken for comparison.

2.5. Urine opioid analysis

Measurement of urine opioids was performed as previously described [21]. "Three supervised urine samples (on Monday, Wednesday, and Friday) were collected from all subjects. Opioids in the urine were analyzed using an enzyme multiplied immunoassay technique. This technique has high sensitivity and specificity (>0.90) for detecting 6 drugs including the methadone, marijuana, phencyclidine, opiates, amphetamines, and cocaine [22]. Polyconsumers were excluded from the study. Urine samples positive for opioids were further processed to distinguish between morphine derivatives such as heroin and codeine. Cut-off points (lower limit of detection), established reference values, were >300 ng/ml for drug or metabolite except for amphetamine where the cut-off point was >1000 ng/ml.

2.6. Sperm chromatin structure assay (SCSA)

Sperm DNA damage was detected with the SCSA as described in our previous study [23]. All the samples were coded and the SCSA were performed by the same person. SCSA was done in 5000 sperm from each of the participants. In brief, sperm samples were diluted to a concentration of 2×10^6 cells/ml with TNE buffer, containing 0.15 mol/l NaCl, 0.1 mol/l Tris and 1 mmol/l ethylenediaminetetraacetic acid (pH 7.4). Then sperm were stained by adding 1.2 ml with the fluorescent dye acridine orange staining solution. Next, the cells were immediately analyzed by Coulter® Epics® XL™ Flow Cytometer. The degree of DNA denaturation was quantified by flow cytometric measurements of the metachromatic shift from green fluorescence (native double strand DNA) to red (single strand DNA) fluorescence.

The red and green fluorescence signals were measured using 675-nm and 525-nm detectors, respectively. The metachromatic shift from green to red fluorescence was calculated by the equation, αt = [red/(red + green) fluorescence], expressed as a percent, and as (COMP) αt, where COMP stands for cells outside the main population. COMP αt was computed as the percent of cells in the population showing an αt value of greater than 26%. DNA fragmentation index (DFI) was determined using ListView software (Phoenix Flow Systems, San Diego, CA).

2.7. Seminal plasma antioxidant capacity

Superoxide dismutase (SOD)-like activity was determined using the inhibition of nitroblue tetrazolium (NBT) reduction by the combination xanthine–xanthine oxidase as described by Zini et al. [24]. The amount of seminal plasma able to diminish the reduction of NBT by 50% was set as one unit of SOD-like activity.

Catalase-like activity was determined by the reduction in concentration of exogenous hydrogen peroxide (H_2O_2) after incubation with the test samples using the method described by Yeung et al. [25]. The amount of seminal plasma able to decrease the concentration of H_2O_2 present in solution by 50% was set as one unit of catalase-like activity.

All laboratory measures were performed in a blinded manner. Of recruited subjects 142 opiate consumer men (group 1) and 146 control subjects (group 2) met study criteria and were selected for analysis.

2.8. Statistical analysis

A sample size of 140 subjects per group provided 85% power to detect a 15% difference in sperm motility between cases and controls. All values were presented as the mean $\pm$ SD, unless otherwise stated. All variables were initially tested for homogeneity and data normality. All sperm parameters were skewed toward the left. Skewed distribution of sperm parameters and abnormally distributed data was normalized by square root or logarithmic transformation as appropriate. Differences in sociodemographic variables between groups were examined with the paired t-test for dichotomous variables and one-way ANOVA test for normally distributed continuous variables. To determine differences in serum hormone values, the repeated measures ANOVA or the nonparametric Friedman ANOVA, followed by the paired t-test were used. The correlation between sperm parameters and opiate consumption with adjustment for several potential confounding factors were also analyzed. Adjustment for confounding factors was performed by putting the demographic (age, BMI, occupational status, educational level, and smoking status) and clinical (serum testosterone, LH, and PRL) covariates into the multivariable logistic regression model. Comparisons of the percentage of denatured DNA measured by SCSA of sperm from both groups were made with the Mann–Whitney U-test. Correlations were determined by Spearman's rank correlation test. Odds ratios (ORs) and 95% confidence intervals (CIs) were computed by an unconditional logistic regression

model. Significant differences were set at P value <0.05. Statistical analysis was carried out by Statistical Package for the Social Sciences (SPSS) version 17.0 (SPSS Inc, Chicago, IL).

3. Results

Tables 1 and 2 demonstrate data obtained after logarithmic transformation of sperm parameters and serum hormone concentrations.

3.1. Testicular volume

The testicular volume was not significantly different when the men in group 1 were compared with control men. However, a reduced testicular volume was found among those men with severe oligoasthenoteratozoospermia (sperm density $<10 \times 10^6$/ml), when compared to the accepted normal value for testicular volume of 15 ml [26].

3.2. Serum hormones

As shown in Table 1, mean serum levels for LH, T, and fT were significantly lower in opium addict subjects than in the control. FSH levels were also lower in opium addicts, although the difference was not statistically significant. Serum levels for other measured hormones were similar for the two groups.

3.3. Sperm parameters

Based on WHO criteria 51 cases (35.9%) and 14 controls (9.6%) had an abnormal sperm concentration ($\leq 20 \times 10^6$/ml) ($P = 0.001$).

Table 1
Frequency distributions of selected variables between cases and controls.

Variables	Cases ($n = 142$)	Controls ($n = 146$)	P value*
Age (years)	34.6 ± 6.7	33.8 ± 6.5	0.24
Age distribution, no (%)			
≤30	36 (25.3)	38 (26.0)	0.76
31–40	62 (43.7)	65 (44.5)	0.33
41–50	44 (31.0)	43 (29.5)	0.24
BMI category, kg/m^2	26.2 ± 2.1	26.6 ± 2.2	0.18
Testicular volume, ml	20.2 ± 2.3	24.2 ± 3.3	0.07
Marital status, no (%)			
Married	86 (60.6)	104 (71.2)	0.01
Never married	22 (15.5)	15 (10.3)	0.03
Divorced, separated or widowed	34 (23.9)	27 (18.5)	0.03
Occupational status, no (%)			
Employed	114 (80.3)	120 (82.2)	0.08
Unemployed	28 (19.7)	26 (17.8)	0.07
Education level			
None	0 (0)	0 (0)	NA
Primary school	54 (38.0)	42 (28.8)	0.01
High school	84 (59.2)	86 (58.9)	0.08
Graduate	4 (2.8)	18 (12.3)	0.004
Serum hormones			
Testosterone (ng/ml)	5.7 ± 1.36	7.4 ± 2.27	0.04
Free testosterone (pg/ml)	134.7 ± 39	153.6 ± 41	0.03
LH (IU/l)	6.2 ± 2.4	7.8 ± 2.2	0.03
FSH (IU/l)	6.2 ± 2.1	7.2 ± 2.4	0.08
PRL (pmol/l)	354 ± 122	365 ± 119	0.09
TSH (mIU/ml)	2.2 ± 1.2	2.1 ± 1.2	0.1
Free thyroxine (pmol/l)	13.7 ± 2.5	14.1 ± 2.6	0.09
Free triiodothyronine (pmol/l)	3.6 ± 1.2	3.5 ± 1.2	0.08
SHBG (nmol/l)	21.6 ± 4.1	22.1 ± 4.3	0.08
Serum biochemistry			
Alanine aminotransferase (IU/l) 31 ± 14	29 ± 12	0.09	
Aspartate aminotransferase (IU/l) 30 ± 14	27 ± 14	0.08	
Alkaline phosphatase (IU/l)	232 ± 48	237 ± 51	0.08
Total bilirubin (mg/dl)	0.8 ± 0.2	0.8 ± 0.2	0.09
Creatinine (mg/dl)	1.1 ± 0.2	1.0 ± 0.4	0.1
Smoking status			
Never	30 (21.1)	48 (32.9)	0.01
Former	32 (22.5)	34 (23.3)	0.07
Current	80 (56.3)	64 (43.8)	0.01
Routes of opiate administration, no (%)			
Smoking	49 (34.5)	–	NA
Snorting	34 (23.9)	–	NA
Ingestion	18 (12.7)	–	NA
Both smoking or snoring and ingestion	17 (12.0)	–	NA
Intravenous injection	24 (16.9)	–	NA
Duration of dependence, month	58.2 ± 34.5	–	NA
Types of opiate, no. (%)			
Crude opiate (Teriak) only	36 (25.3)	–	NA
Refined opiate (Shireh) only	32 (22.5)	–	NA
Both crude and refined opiate	38 (26.8)	–	NA
Heroin	36 (25.3)	–	NA
Opiate use per day, Nokhod	2.7 ± 1.2	–	NA
Heroin use per day, mg	8.2 ± 3.3	–	NA

Keys: LH, luteinizing hormone; FSH, follicle stimulating hormone; PRL, prolactin; TSH, thyroid stimulating hormone; SHBG, sex hormone binding globulin; NA, not applicable.
* Derived from two-sided χ^2-test or Student's t-test.

Table 2
Routine semen parameters and SCSA results in participants.

Variables	Cases ($n = 142$)	Controls ($n = 146$)	P value*
Semen variable			
Ejaculate volume (ml)	2.6 ± 1.2	2.8 ± 1.2	0.27
Total sperm/ejaculate ($\times 10^6$)	48.7 ± 12.7	188.2 ± 31.7	0.001
Sperm concentration ($\times 10^6$/ml)	22.2 ± 4.7	66.3 ± 8.3	0.002
Motility (% motile)	38.2 ± 7.2	61.8 ± 5.1	0.001
Normal forms (%)	24.8 ± 4.3	63.7 ± 4.4	0.001
Immunobead binding test			
IgG (%)	4.17 ± 10.24	4.14 ± 11.02	0.21
IgA (%)	3.32 ± 6.34	3.27 ± 6.28	0.28
% Acrosome reaction	24 ± 8	35 ± 11	0.02
Seminal plasma antioxidant status			
Catalase-like activity (U/ml)	316 ± 17	371 ± 42	0.003
SOD-like activity (U/ml)	38.4 ± 1.4	49.3 ± 2.2	0.002
DFI, mean %	36.4 ± 3.8	27.1 ± 2.4	0.004

Key: SOD, superoxide dismutase.
* Derived from two-sided χ^2-test or Student's t-test.

As shown in Table 2, mean sperm count, mean total sperm count, mean percentage of normal forms, and mean sperm motility were significantly lower in opium addict subjects than in the control.

3.4. Immunobead binding test

Binding of antisperm antibody of the IgG and IgA types were not influenced by opiate consumption (Table 2).

3.5. Acrosome reaction test

The mean acrosome reaction was 24 ± 8% and 31 ± 11% in groups 1 and 2, respectively ($P = 0.02$).

3.6. Seminal plasma antioxidant capacity

Significantly lower concentrations of SOD-like (38.4 ± 1.4 and 49.3 ± 2.2 U/ml) and catalase-like (316 ± 17 and 371 ± 42 U/ml) activities were seen in cases compared with control men ($P = 0.002$ and $P = 0.003$, respectively) (Table 2).

3.7. %DFI

Spermatozoa from cases had more abnormal chromatin than their control counterparts ($P = 0.007$) (Table 2). Men in group 1 had a significant increase in the percent DFI compared with group 2 (36.4 ± 3.8 vs. 27.1 ± 2.4%, Mann–Whitney U-test, $P = 0.004$).

3.8. Correlations analyses

These data are shown in Table 3. There was a negative correlation between opium consumption and normal sperm parameters, and a positive correlation between opium consumption and abnormal parameters. We also observed a significant negative correlation between the duration of opiate consumption and sperm parameters. Based on Spearman's rank correlation test each sperm parameter was significantly negatively correlated with duration of drug dependence. Drug dependence duration was also significantly positively correlated with %DFI representing sperm. Seminal plasma catalase and SOD like activities were significantly correlated with drug dependence duration, types of opiate consumed, amounts of opiate consumed and routes of opiate administration. Types of opiate consumed and rout of administration also had significant correlations with sperm parameters, seminal plasma antioxidant capacity, and %DFI. The worst effects were related to the heroin and intravenous injection (for details see Table 3).

4. Discussion

Our study detected significant association between opiate consumption and impaired sperm parameters and increased sperm chromatin damage as measured by the DNA fragmentation index. In addition, the motility and morphology of the sperm in the cases, apart from being significantly lower than the controls, are also under the WHO normal values. There was a dose-related association between opiate consumption and sperm DNA damage and impaired semen parameters, with the group with high intake having the poorest semen parameters and highest %DFI. Men in group 1 had also significantly decreased serum T and fT levels. Despite the presence of hypotestosteronemia and impaired spermatogenesis, serum levels of LH and FSH did not increase. These findings are consistent with previous studies demonstrating that opiate consumption can result in a hypogonadotropic hypogonadism state [6]. When we examined the relationship adjusting for age, BMI, smoking status, and serum testosterone levels, the observed significant associations remained unchanged. Usually opiate users are polyconsumers. We used the enzyme multiplied immunoassay technique for urine testing, which has high sensitivity and specificity for the 6 drugs tested: marijuana, cocaine, opiates, phencyclidine, amphetamines, and methadone. Patients who were polyconsumers according to urine toxicology test were excluded from the study. However, for other substance abuse such as ecstasy we solely relied to patients' claims. It has been documented that excess ROS could result in structural and functional sperm damage [27]. Arana et al. reported that, drug addicts have significantly lower salivary glutathione peroxidase and reductase activities than controls [28]. In present study, group 1 had significantly lower seminal plasma antioxidant activity which results in oxidative stress. Oxidative stress may lead to DNA damage in human spermatozoa [29]. Our finding of increased %DFI may be due to an imbalance between the antioxidant capacity in seminal plasma and the ROS production. In addition this finding may implies that, the impaired semen quality and male infertility found in cases appear to be due not only to drug influences in the hypothalamus and pituitary gland, but also to direct effect on sperm DNA integrity. It may also explain why semen with impaired quality can be present in heroin addicts with both normal and abnormal serum gonadotropin concentrations.

The problem of infertility is a phenomenon encountered in 8% of married couples in Iran [30]. A decrease in male fertility and increase in abnormal semen parameters has occurred over recent years. One of the main reasons for the increase in impaired semen parameters is believed to be increasing exposure to toxicants in the environment. The causative agents may be chemical materials, stress, ionizing radiation, as well as substance abuse [31,32].

Table 3
Correlation between semen parameters and study variables.

Variables	Sperm concentration			Sperm motility			Sperm with normal forms			SOD-like activity			Catalase-like activity			%DFI		
	Mean *r*	95% CI	*P* value	Mean *r*	95% CI	*P* value	Mean *r*	95% CI	*P* value	Mean *r*	95% CI	*P* value	Mean *r*	95% CI	*P* value	Mean *r*	95% CI	*P* value
Age	−0.23	−0.17 to −0.48	0.02	0.18	−0.32 to 0.46	0.33	0.16	−0.31 to 0.47	0.34	0.22	−0.31 to 0.45	0.52	0.28	−0.34 to 0.45	0.32	0.17	−0.28 to 0.32	0.44
Duration of dependence	−0.72	−0.37 to −0.86	0.001	−0.57	−0.36 to −0.68	0.01	−0.48	−0.24 to −0.64	0.01	−0.61	−0.37 to −0.78	0.001	−0.62	−0.35 to −0.79	0.001	−0.74	−0.56 to −0.84	0.001
Serum hormones																		
Total testosterone	0.19	−0.28 to 0.39	0.27	0.16	−0.27 to 0.38	0.42	0.24	−0.31 to 0.42	0.36	0.26	−0.29 to 0.44	0.52	0.18	−0.33 to 0.36	0.25	0.22	−0.37 to 0.40	0.28
Free testosterone	0.24	−0.37 to 0.42	0.31	0.20	−0.28 to 0.37	0.28	0.21	−0.34 to 0.36	0.38	0.23	−0.32 to 0.46	0.37	0.17	−0.31 to 0.33	0.47	0.19	−0.34 to 0.40	0.37
LH	0.22	−0.32 to 0.46	0.52	0.20	−0.31 to 0.44	0.61	0.16	−0.29 to 0.34	0.47	0.18	−0.31 to 0.46	0.29	0.20	−0.32 to 0.44	0.54	0.18	−0.30 to 0.47	0.63
FSH	0.21	−0.31 to 0.42	0.51	0.19	−0.32 to 0.38	0.58	0.23	−0.33 to 0.47	0.41	0.22	−0.32 to 0.42	0.30	0.22	−0.36 to 0.42	0.51	0.23	−0.31 to 0.47	0.62
SHBG	0.26	−0.24 to 0.62	0.31	0.24	−0.28 to 0.41	0.50	0.25	−0.36 to 0.38	0.54	0.17	−0.29 to 0.38	0.44	0.16	−0.28 to 0.44	0.73	0.26	−0.38 to 0.47	0.54
Types of opiate																		
Crude opiate (Teriak) only	−0.47	−0.28 to −0.72	0.007	−0.42	−0.24 to −0.62	0.006	−0.41	−0.25 to −0.65	0.006	−0.48	−0.29 to −0.68	0.005	−0.44	−0.22 to −0.66	0.007	−0.46	0.24 to 0.64	0.007
Refined opiate (Shireh) only	−0.54	−0.37 to −0.72	0.005	−0.56	−0.34 to −0.68	0.004	−0.55	−0.32 to −0.69	0.004	−0.49	−0.29 to −0.63	0.004	−0.54	−0.29 to −0.73	0.005	−0.56	0.32 to 0.67	0.005
Both crude and refined opiate	−0.62	−0.45 to −0.78	0.003	−0.64	−0.44 to −0.77	0.003	−0.67	−0.46 to −0.78	0.003	−0.69	−0.43 to −0.80	0.003	−0.64	−0.42 to −0.76	0.003	−0.67	0.41 to 0.76	0.003
Heroin	−0.75	−0.51 to −0.89	0.001	−0.76	−0.44 to −0.88	0.001	−0.76	−0.45 to −0.89	0.001	−0.71	−0.47 to −0.84	0.001	−0.73	−0.45 to −0.86	0.001	−0.77	0.51 to 0.89	0.001
Routes of opiate administration																		
Smoking	−0.52	−0.35 to −0.68	0.006	−0.54	−0.34 to −0.67	0.005	−0.51	−0.37 to −0.66	0.006	−0.52	−0.35 to −0.68	0.005	−0.49	−0.34 to −0.66	0.005	−0.50	0.32 to 0.69	0.004
Snorting	−0.48	−0.33 to −0.64	0.006	−0.46	−0.36 to −0.64	0.007	−0.50	−0.38 to −0.68	0.006	−0.52	−0.34 to −0.67	0.004	−0.53	−0.32 to −0.69	0.006	−0.49	0.37 to 0.67	0.006
Ingestion	−0.72	−0.54 to −0.88	0.002	−0.76	−0.56 to −0.87	0.003	−0.71	−0.56 to −0.87	0.003	−0.74	−0.55 to −0.86	0.002	−0.72	−0.53 to −0.84	0.003	−0.77	0.54 to 0.90	0.001
Both smoking and snoring	−0.46	−0.28 to −0.64	0.006	−0.49	−0.32 to −0.68	0.005	−0.50	−0.36 to −0.68	0.005	−0.51	−0.30 to −0.68	0.004	−0.48	−0.31 to −0.63	0.006	−0.48	0.32 to 0.66	0.006
Intravenous injection	−0.80	−0.62 to−0.91	0.001	−0.81	−0.64 to −0.92	0.001	−0.79	−0.66 to −0.88	0.001	−0.82	−0.68 to −0.92	0.001	−0.82	−0.62 to −0.90	0.001	−0.84	0.71 to 0.93	0.0006
Opiate use per day > 1 Nokhod	−0.74	−0.52 to −0.81	0.001	−0.73	−0.54 to −0.82	0.001	−0.76	−0.55 to −0.84	0.001	−0.81	−0.63 to −0.91	0.003	−0.81	−0.64 to −0.90	0.002	−0.81	0.72 to 0.90	0.0004
Heroin use per day > 5 mg	−0.81	−0.63 to −0.92	0.001	−0.79	−0.62 to −0.91	0.002	−0.77	−0.65 to −0.84	0.001	−0.79	−0.66 to −0.91	0.002	−0.79	−0.61 to −0.90	0.003	−0.82	0.70 to 0.90	0.0005

Keys: LH, luteinizing hormone; FSH, follicle stimulating hormone; SHBG, sex hormone binding globulin.

Substance abuse is a growing social and medical problem both in developed and developing countries. The National Survey of Drug Use and Health (NSDUH) conducted in United States indicated that illicit drug use is prevalent among men of reproductive age [33]. Among men in the age groupings of 26–34, 35–49, and 50 years and older, use of any illicit drug in 2008 was 24.6%, 14.5%, and 7.8%, respectively. The illicit drugs which have been reported to adversely affect male fertility are marijuana, methamphetamines, cocaine, opioid narcotics, and anabolic-androgenic steroids [12]. When assessing infertility, it is important to ask about drug use. In clinical practice we frequently encounter men with oligoasthenoteratozoospermia (OAT) who are opiate consumers, and cessation of these substances often results in improved semen quality. The present study supports the concept that opioids may impair male fertility capacity at multiple sites. A variety of demographic characteristics (age, BMI, occupational status, educational level, and smoking status) and clinical covariates (serum testosterone, LH, and PRL) are known to be correlated with fertility status [30]. To control for any impact of possible confounding on the main effect of the opiate consumption, we used multivariate analysis.

There are several limitations to the present study. It is difficult to examine and control for multiple opioid interactions as many persons who consume opiates are frequently not just taking one type of opiate. Due to limited resources, we only examined the patients' urine for 6 drugs which are commonly abused. In addition we did not address the participants' paternity. Impaired semen quality does not translate into an impaired paternity. The wide age range of participants included in the study (20–50 years) is another limitation. The age may affect the effects on seminal parameters independently of the opium consumption.

5. Conclusion

We indicated for first time the adverse effects of opiate consumption on sperm production and sperm chromatin structure in humans.

Conflict of interest

None declared.

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