

The Effect of Heroin Addiction on Human Sperm Parameters, Histone-to-Protamine Transition, and Serum Sexual Hormones Levels

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Purpose: To investigate the effects of heroin on sperm parameters, histone-to-protamine transition ratios in mature sperm, and serum reproductive hormone levels in active heroin users.

Materials and Methods: Semen and blood samples were collected from 25 men who used only heroin for at least 12 months and the same number healthy men who did not use any drugs and did not suffer from infertility problems. Computer-based analysis, Aniline blue staining, and hormonal assessment were performed to provide valuable new information on the relationship between addiction and semen profile and serum reproductive hormone levels.

Results: Our finding showed that semen pH (7.8 vs. 7.75), sperm motility ($42.93 \pm 3.89\%$ vs. $68.9 \pm 2.68\%$), and viability ($73.27 \pm 3.85\%$ vs. $86.48 \pm 1.05\%$), and sperm histone replacement abnormalities ($32.33 \pm 10.89\%$ vs. $5.56 \pm 0.85\%$) were significant differences in addicted group vs. non-exposed ones ($P \leq .05$). In addition, serum sex hormone levels were not significantly differed between groups. There was a correlation between the amount of daily heroin consumption and LH level. We also observed that duration of drug dependence is correlated with sperm abnormalities.

Conclusion: We concluded that heroin consumption affect sperm maturities such as histone-to-protamine ratio and impair semen profile in general and particularly sperm morphology and motility. Heroin may be considered as one of the idiopathic male infertility reason.

Keywords: heroin addiction; Histone-to-Protamine transition; sexual hormones; sperm parameters

INTRODUCTION

Addiction as a medical problem in the world is developing among young people⁽¹⁾. Based on formal statistics there are more than 2 million addicted people in Iran that one-third of them use heroin⁽²⁻⁴⁾. Prescription opioid narcotics such as heroin can result in intoxication, medical, and social problems⁽⁵⁾. Toxins are considered as important risk factors in the reproductive biology⁽⁶⁾. However, prescription opioid narcotics as the well-known toxins, may be interfere with male fertility⁽⁷⁾ and sperm parameters, specifically sperm motility, decrease with the use of heroin⁽⁸⁾. Use of illicit drugs appears to have a negative impact on fertility, though more in-depth research in this area is required to make a clear link.

Previous studies showed that addiction has decreased

male fertility capacity with damage to sperm motility and normal morphology^(9,10). Also, it could retard pre-implantation development and induces apoptosis in embryos of addicted mice⁽¹¹⁾. Accurate deoxyribonucleic acid (DNA) packaging is one of the most important factors in the health of sperm motility and morphology. Spermatozoa DNA is about ten times more compact than the somatic cells⁽¹²⁾. Chromatin compaction in mature sperm is facilitated with histone to protamine replacement. In human mature sperm, the histone-to-protamine substitute rate is between 80 to 85%. Some reports have demonstrated that cigarette smoking can affect this replacement and result in abnormal sperm morphology and motility⁽¹³⁾. Crack and heroin addiction have decreased sperm motility and normal morphology in mice^(14,15).

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Table 1. Demographic and seminal data in the study population

Parameter	Non-exposed group Mean \pm SD (Min-Max/Median)	Addicted group Mean \pm SD (Min-Max/Median)	P-value
Demographic data			
Age (year)	34.81 \pm 1.53	33.15 \pm 1.85	.51
BMI (kg/m ²)	25.85 \pm .70	22.30 \pm .36	.001
Smoking (yes/no)	(4/25)	(25/0)	.001
Duration of dependence (year)	-	(2-32/12)	
Duration of Heroin consumption (year)	-	(1-25/5)	
Heroin use (mg/day)	-	(0.5-5/1)	
Seminal parameters			
Semen physical parameters			
Volume (mL)	4.1 \pm .45	3.09 \pm .35	.08
Agglutination	(0-2/0)	(0-1/0)	0.88
Aggregation	(0-2/0)	(0-3/0)	.57
Semen pH	(7.5-8/7.8)	(7.2-7.9/7.75)	.001
Sperm concentration ($\times 10^6$ /mL)	156.77 \pm 35.56	158.14 \pm 40.34	.98
Viability (%)	86.48 \pm 1.05	73.27 \pm 3.85	.01
Sperm motility (%)			
Total	68.9 \pm 2.68	42.93 \pm 3.89	.001
Progressive	38.35 \pm 3.5	22.69 \pm 3.32	.003
Abnormal morphology (%)			
Total	6.67 \pm 0.67	88.12 \pm 5.1	.001
Head	3.67 \pm 0.88	74.62 \pm 3.47	.001
Tail	3 \pm 1	13.5 \pm 2.51	.037
Histone-to-protamine ratios	5.56 \pm 0.85	32.33 \pm 10.89	.05
Appearance of Blood cells in semen ($\times 10^6$/mL)			
WBC	1.48 \pm 0.37	9.26 \pm 1.86	.001
RBC	(0-0.9/ .5)	(0-4.1/ .5)	.2
Appearance of other cells in semen ($\times 10^6$/mL)			
Germ	(0-1/0)	(0-1/0)	.28
Round	(0-7.9/1.5)	(0-11.1/2)	.86

On the other hand, the amount of serum sexual hormones is correlated with the usage of illicit drugs. There is controversy in hormonal levels in human studies because of opiate administration route, types of opiate, the amount of usage per day, last time of opiate usage, and history of addiction^(9,10). However, in animal studies, many reports have described the correlation between decreased reproductive hormones and addiction^(16,17). The aim of this study was to evaluate the effect of heroin on semen quality such as sperm motility and morphology, histone-to-protamine transition, and serum sexual hormone levels among active heroin users. Since the correlation among addiction, semen profile and sperm maturity were unknown, we focused on this subject. Also, we assessed some new sperm parameters.

MATERIALS AND METHODS

Study population

The medical ethics committee of Iran University of Medical Science approved this study (code: IR.IUMS.rec.1394.9211313202). The study requirements were carefully explained to participants and all subjects were interviewed after written informed consent, which was conducted in accordance with the Declaration of Helsinki. The data on personal information, history of addiction, and medical status were collected via a structured questionnaire.

Twenty-five 20-50-year-old men with normal body mass index (BMI) were screened for eligibility addiction in the addicted group. 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 (DS-MO-V) criteria for addiction. Based on their medical records and questionnaires they were just using heroin

for at least 12 months and were not taking other drugs during this period. Since we did not have any information about their infertility situation before addiction, it was considered as a limitation of the study. In this study we could not follow up the members of addicted group during the biological sample collection. Non-exposed group consisted of 25 healthy age-BMI-matched male volunteers without any addiction history. They were healthy male partners of married couples without illicit drug consumption who had attended the Shahid Akbar-Abadi Obstetrics and Gynecology Hospital of the Iran University of Medical Science for female infertility consultation. Subjects with medical problems associated with subfertility were excluded from our study. Other exclusion criteria were taking of any other illicit-drug in at least 1 year ago in the addicted group and taking of any type of drugs in non-exposed ones.

Inclusion and exclusion criteria

Volunteers were participated in the study if they were between 20 and 50 years. None of the participants had known medical or surgical condition that could influence their fertility. In non-exposed group participants had normal semen profile who had attended the Shahid Akbar-Abadi Obstetrics and Gynecology Hospital (Tehran, Iran) for female infertility consultation. In this group, subjects with a history of any genitourinary surgery, azoospermia, epididymo-orchitis, varicocele, cryptorchidism, and alcohol consumption were excluded from study. In addicted men, all members used only heroin at least for one year. Men who consumed heroin and other drugs simultaneously were excluded from study. Other exclusion criteria was normal body mass index (18.5 - 25).

Table 2. Partial correlation between duration of dependence, the amount of heroin consumption and some study variables.

Parameters	Correlation	P-value
Duration of drug dependence (year)		
Semen pH	-0.464	.007
Sperm total motility (%)	-0.370	.037
LH (mIU)	-0.428	.018
WBC (%)	0.396	.025
Duration of heroin dependence (year)		
sperm abnormal morphology (%)	0.996	.05
The amount of heroin consumption (mg/day)		
pH	- 0.334	.05
LH (mIU)	0.408	.025

These correlations were adjusted for age, BMI, and cigarette smoking status.

Semen collection and computer-assisted sperm analysis (CASA) analysis:

Semen was collected in sterile containers from participants by masturbation after 2 to 5 days of sexual abstinence. Samples incubated at 37 °C for at least 30 minutes and analyzed by semen collection and computer-assisted sperm analysis (CASA) according to 2010 World Health Organization (WHO) criteria for semen volume, pH, viscosity, agglutination, aggregation, the appearance of other cells in semen such as blood cells, round cells, and germinal ones, sperm concentration and their vitality, sperm morphology and sperm motility.

Aniline blue staining

Aniline blue was used to detect the immature spermatozoa with excessive histones. Briefly, 10 µl of washed spermatozoa were spread onto the slides. Dried smears were fixed in 3% buffered glutaraldehyde in 0.2 M phosphate buffer (pH 7.2) for 30 minutes. Slides were stained with 1% aniline blue mixed with 4% acetic acid (pH 3.5) for 5 minutes. Then the staining slides were washed in running water for at least 3 minutes and dehydrated in a graded ethanol for 2 minutes in each step. Finally, slides were cleared by xylene for at least 30 minutes and were mounting by a drop of Entellan and were dried overnight at room temperature. At least 200 sperm cells per slide were evaluated under light microscopy with an objective lens (×100) and the percentage of stained sperm heads was calculated.

Hormone assessment

Peripheral blood of participants (5mL) was collected in the morning after eating breakfast and before semen collection. Samples centrifuged for 10 min at 4°C and

3000 rpm. The serum concentrations of estradiol (E2), luteinizing hormone (LH), follicle stimulating hormone (FSH), and prolactin (PRL) were determined by ELISA kits and ELISA reader. Serum testosterone (T) levels were measured by Chemiluminescent Enzyme Immunoassay.

Statistical analysis

Statistical analysis was done with a statistical software package (Ver. 16.0, Chicago, SPSS Inc.). The parametric distribution was evaluated with Kolmogorov-Smirnov test. The differences between groups were determined by independent-samples *t*-test and Man-Whitney U. The partial correlation analyses were performed between heroin consumption and other parameters. *P*-value ≤ .05 was proposed to be significant.

RESULTS

Demographic information

Demographic information of study population is shown in **Table 1**. There was no significant difference in the mean age between 2 groups. Although BMIs were in the normal range in both groups, this parameter in the addicted men ($22.3 \pm 0.36 \text{ kg/m}^2$) was significantly lower than the non-exposed group ($25.85 \pm 0.7 \text{ kg/m}^2$) ($P \leq .01$). All subjects in the addicted group were smoker, so there was a statistical difference between groups ($P \leq .01$).

Semen Analysis

Although semen volume and its viscosity, agglutination, aggregation, sperm concentration, the appearance of germ and round cells in semen were the same between groups, the amount of white blood cell (WBC)

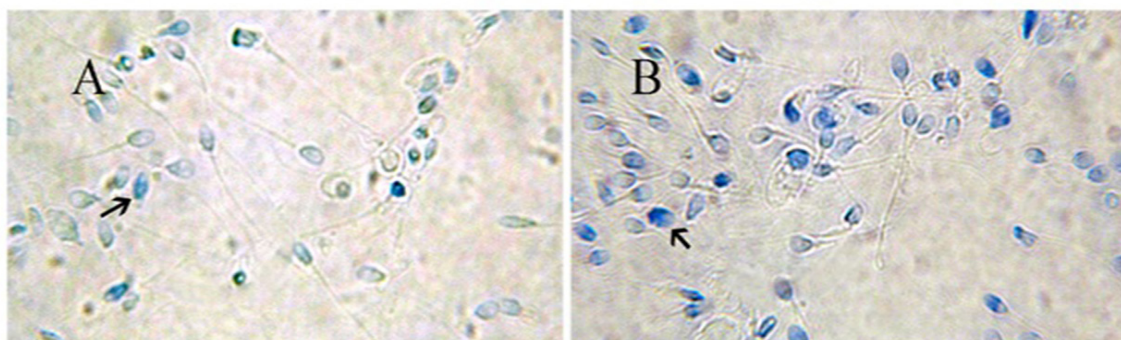


Figure 1. Aniline blue staining for detection of the histone-to-protamine ratio in sperm nucleus. The sperm cells with increased histone-to-protamine ratios are shown by arrow in (A) healthy and (B) addicted men. Positive staining shows the nucleus packaging abnormality in sperms.

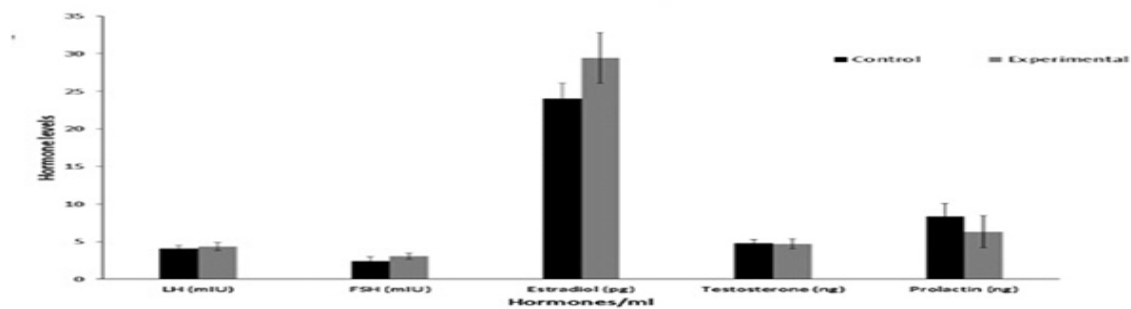


Figure 2. Comparison amount of reproductive hormone in serum of addicted and non-exposed men. Serum levels of LH, FSH, estradiol, testosterone and prolactin were not significant differences between groups ($p \geq .05$).

in semen and abnormal morphology were statistically different between addicted group and non-exposed ones (9.26 ± 1.86 vs. $1.48 \pm 0.37 \times 10^6/\text{mL}$, 88.12 ± 5.1 vs. $6.67 \pm 0.67\%$ respectively; $P \leq .01$). Semen pH ($7.2-7.9$ vs. $7.5-8$) and sperm viability and motility (73.27 ± 3.85 vs. $86.48 \pm 1.05\%$ and 42.93 ± 3.89 vs. $68.9 \pm 2.68\%$ respectively) in the addicted group were significantly lower than non-exposed ones ($P \leq .01$) (Table 1).

Sperm nuclear histone-to-protamine ratios

The sperm cells with increased histone-to-protamine ratios are shown by the arrow in non-exposed (Figure 1.A) and addicted group (Figure 1. B). There was a significant higher histone replacement abnormality in addicted group than non-exposed ones (32.33 ± 10.89 vs. $5.56 \pm 0.85\%$; $P < .01$) (Table 1).

Sexual hormones analysis

As shown in Figure 2, mean serum levels of LH (4.36 ± 0.4 vs. 4.72 ± 0.53 mIU/mL), FSH (3.07 ± 0.59 vs. 3.27 ± 0.41 mIU/mL), E2 (24.06 ± 2.01 vs. 30.87 ± 3.95 pg/mL), PRL (10.25 ± 1.68 vs. 6.71 ± 1.03 ng/mL), and T (4.42 ± 0.48 vs. 4.43 ± 0.69 ng/mL) revealed no significant difference between addicted and non-exposed groups.

Correlations analysis

Based on partial correlation test semen pH ($r = -.464$, $P = .007$), sperm motility ($r = -.370$, $P = .037$), and the amount of serum LH ($r = -.428$, $P = .018$) were significantly negatively correlated with duration of drug dependence. We also observed a significant positive correlation between WBC and time of drug dependence ($r = 0.396$, $P = .025$). Duration of heroin dependence was related to sperm abnormal morphology ($r = 0.996$, $P = .05$). The amount of daily heroin consumption correlated to serum LH level ($r = 0.408$, $P = .025$) when adjusted for age and BMI and cigarette smoking (Table 2).

DISCUSSION

Considering the role of recreational heroin consumption as one of the potential risk factors of male infertility, we investigated the effect of heroin use on sperm parameters, histone-to-protamine transition ratio, and serum sexual hormone levels as well as these factors' possible correlations in participants. However, lack of information about their infertility situation before addiction was considered as a limitation of the study. As another limitation, we could not follow up the participants during the biological sample collection.

This was the first study that evaluated some clinical aspects of male reproductive parameters in active heroin users. So, semen and blood samples were collected from 25 heroin users and 25 healthy men. Then semen analysis, aniline blue staining, and hormones assessment were performed to provide valuable new information on the relationships of addiction and semen profile and serum reproductive hormone levels. Our data determined a significant association between heroin consumption and impaired semen parameters and histone-to-protamine transition ratios. Confounding factors in this study were controlled by partial analysis.

Very few studies have done to find out the direct effect of heroin on male infertility. Main obstacles on the way of human studies can be legislation and ethical considerations.

In the present study, although BMI in the addicted group was in normal range, it was significantly lower than in the non-exposed one. Our finding was similar to other previous studies who mentioned illicit drug use decreases body weight in addicted animal^(14,17,18) and human⁽¹⁹⁾. The average BMI may affect spermatogenesis. However, partial correlation results showed that heroin can be more effective than BMI in semen parameters. In this study, alteration of semen quality such as reducing pH and increasing WBC were observed. We proposed that WBC in semen can alter semen microenvironment and affect semen pH and semen acidification may affect sperm viability and motility. Increasing blood cells in the semen were observed in all addicted men, so we were not able to control confounding factor. Previous studies had revealed that the presence of WBCs in semen which in association with ROS, may manifest as male factor infertility⁽²⁰⁻²²⁾. This study showed that duration of dependency was significantly negatively associated with sperm motility. Due to the presence of opiate receptors in different regions of the spermatozoa, reduced viability and motility and abnormal sperms can be caused directly by heroin because activation of these receptors leads to an anti-motility effect. Based on previous studies, the opioid system may control reproductive function in the central nervous system (CNS), the pituitary gland, and the testis, exerting a direct action on the spermatozoa. An adequate level of opiate helps sperm motility but this effect depends on opiates' concentration⁽²³⁾. In addition, our previous studies showed that cytoskeletal proteins in the sperm tail such as testis-specific gene antigen10 (Tsga10), and ion channel proteins (Catsper 1-4) in sperm can alter

sperm motility^(6,14) and antioxidants can help sperm parameters on the inappropriate condition⁽²²⁾. Our study indicated that the heroin consumption was associated with a significant increase in head abnormality and histone-to-protamine transition ratios. Amini et al. (2014) showed that sperm abnormality increased following Kerack administration in mice⁽¹⁴⁾. Safarinejad et al. (2013) reported increased sperm DNA damage in opiate users. They suggested that opiate has a negative effect on DNA integrity through two pathways: first, the hypothalamus and pituitary gland, and second, direct effect on sperm DNA integrity⁽⁷⁾. Our findings are similar to these previous studies^(7,13,14). Although the molecular mechanisms of opiate-induced nuclear abnormality in human sperm are under investigation, mice model correlated the chromatin damage with semen quality following ethanol consumption⁽²⁴⁾ or exposure to cigarette smoke⁽²⁵⁾. Considering the importance of histone-to-protamine transition in the DNA packaging, each abnormality in this process can be associated with subfertility or infertility in human^(13,26). We proposed that in a similar way, opiate may impair the nuclear integrity of sperm.

Our study showed serum sex hormone levels were not significantly different between groups. Correlations demonstrated that addiction and the amount of heroin consumption correlated with LH levels in serum. Previous studies demonstrated that opioid narcotics can cause a defect in the hypothalamic-pituitary-testicular axis^(9,17). Also, the effect of opiate drugs on the amount of serum sexual hormones is also one of the controversial topics in these studies. Cushman (1973) reported Testosterone level unchanged in heroin users⁽²⁷⁾. In contrast, a number of studies found a reduction of plasma sexual hormone levels in active opiate addicted men^(28,29). Also, Yilmaz et al. (1999) suggested chronic morphine exposure significantly decreased serum testosterone and LH levels, but not FSH release in male addicted rats⁽¹⁷⁾. Differences in sampling lag time (since the last time of drug administration), lifestyle could be the main factors in this heterogeneous data. Mirin's team documented just 10 mg of heroin can cause a fall in the plasma LH and testosterone levels immediately and 4 hours later respectively⁽³⁰⁾. Since time management was possible in animal studies, they showed a significant decline in the amount of serum sexual hormones^(18,31). In addition, reproductive hormone levels may be related to opioid-mediated PRL release. Some studies have revealed the increased prolactin levels in rats who treated with opioids⁽³²⁾, and in heroin users following intravenous self-administration of heroin⁽³³⁾. Ellingboe et al. (1980) have shown regular use of heroin leads to tolerance the acute prolactin response⁽³³⁾. Based on these data, the male reproductive hormone profile in serum is a time-dependent factor and cannot be a reliable factor for fertility situation⁽³⁴⁾.

CONCLUSIONS

In summary, the present study showed that heroin consumption alters semen parameters and histone-to-protamine transition ratios in addicted men. We also showed a significantly negative correlation between heroin administration and semen parameters. However, it may increase our understanding of the effect of drugs and toxins on male infertility.

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CONFLICT OF INTEREST

The authors report no conflicts of interest.

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