http://jbcp.shahed.ac.ir 10.22070/jbcp.2019.4722.1120

Open Access Research Article

Journal of Basic and Clinical Pathophysiology
Print ISSN: 2322-1895, **Online ISSN:** 2345-4334
Vol.7, No.2, December 2019

A Semiannual Publication of
Shahed University Press

Interaction of sulpiride with morphine in induction of infertility in male rat

Mahdiyeh Jafarpour Fard [1], Manizheh Karami[2]*, Mohammadreza Jalali Nadoushan[3]

1. Department of Biology, Faculty of Basic Sciences, Shahed University, Tehran, Iran.
2. Department of Biology, Faculty of Basic Sciences, Shahed University, Tehran, Iran.
3. Department of Pathology, Faculty of Medicine, Shahed University, Tehran, Iran.

Abstract

Background and Objective: Male fertility depends on the proper function of a complex system of organs. Harmful effects of morphine on male reproduction and fertility are well documented. Dopamine facilitates sexual behavior in different animal species. Antagonizing of dopamine D2 receptors with sulpiride was aimed to investigate the interaction of dopaminergic system with morphine-induced infertility.

Materials and Methods: A total of 48 adult male Wistar rats with a weight range of 220-320 g were used. In the first category, morphine (5 mg/kg) was injected i.p. The next groups received sulpiride (1-4 mg/kg) alone and prior (20 min) to morphine (5 mg/kg). The control group received only saline (1 ml/kg). All rats were sacrificed under deep anesthesia after one week. Their testicles were collected for examination. LH, FSH and testosterone were measured in the sera. Data were analyzed by ANOVA test.

Results: Significant decreases in dimensions of testicles were observed in those rats that received morphine or sulpiride prior to the morphine. Seminiferous tube destruction was observed in morphine group and in the group that received sulpiride prior to morphine; destruction was increased with increasing dose of sulpiride. Significant decrease of serum testosterone level was observed in rats receiving morphine and a high dose of sulpiride. Significant increase in serum testosterone level was observed in the group receiving sulpiride prior to morphine.

Conclusion: It seems that dopamine has an interacting effect with morphine on induction of male infertility and its mechanism is probably carried out through dopamine D2 receptors.

Keywords: Sulpiride, Dopamine, Infertility, Morphine, Male rat

1. Introduction

Fertility is one of the most important features of the family and infertility can be considered as a major challenge because it has social and economic consequences and costs. The decline in fertility and the increase in infertile couples in developed countries is a serious issue. Infertility has also increased in other human societies around the world. Infertility is not only a female problem, but also male factors are involved. It is nowadays believed that couples play nearly the same role in the causes of infertility (1-4). Male fertility depends on many factors *e.g.* the proper secretion of gonadotropins and the balance between androgens and other topical hormones necessary for spermatogenesis. In addition to all these factors, other environmental factors such as drug abuse should be included. Morphine abuse through central and environmental mechanisms damages the functioning of the reproductive system. Administration of morphine to the opioid system significantly reduces sex hormone levels, spermatogenesis, and adult sperm count in male rats (2). The effects of morphine on male reproduction and fertility have been well elucidated and it should be notified that among the mechanisms involved in the drug abuse, the dopamine dependence pathway

Revised: September 2019**, Accepted:** November 2019, **Available online:** December 2019
***Corresponding Author:** Manizheh Karami **E-mail:** karami@shahed.ac.ir

mechanism is highlighted and also linked to this issue. Dopamine in the brain as a neurotransmitter plays an important role in controlling the endocrine system and sexual behaviors. Dopaminergic neurons are located in 17 different A1-A17 groups in different brain regions. The cell body of these neurons is mainly located in the ventral tegmental area of the midbrain and the hypothalamus and their axons are sent to different areas of the brain. These neurons play an important role in controlling the endocrine system (5). The presence of dopamine receptors in the reproductive organs of rat and other mammalian sperms is an evidence of the role of dopamine in reproduction and fertility (6,7). Dopamine acts as a modulator of sperm motility, vitality, and physiological motility. Dopamine is therefore a modulator involved in the control of *in vivo* fertilization (6) and has a protective effect on sperm (7). Findings suggest that stimulation of peripheral dopamine receptors in seminal vesicles may play a role in semen release and tissue contraction. However, the mechanism of action of peripheral dopamine receptors in the male reproductive tract has not yet been elucidated (8). Sulpiride is a D2 dopamine receptor antagonist that has clinical application as well. In the present study, blockade of dopamine D2 receptors by the aid of sulpiride has been aimed to investigate its interaction with morphine.

2. Materials and Methods

2.1. Animals and experimental procedure

The animals in this experimental study were male Wistar rats, housed in autoclaved cages at standard conditions at the Institutional Laboratory of Animal Care. The number of animals in each cage was two and was monitored during the test stages for nutrition and other environmental factors. The cages were cleaned once every two days and their water and food were monitored. Specific times were scheduled according to the plan and after passing the laboratory animal skills training course (under the supervision of an advisor) and were randomly assigned to different groups to receive the medication. Then, 48 adult male Wistar rats were used for the experiment and were randomly divided into eight groups of six after one week of adaptation to environmental conditions. Before the injection of the drug, the rats were weighed with an animal scale. One week after injection, each group was re-weighed and anesthetized using an anesthetic combination (ketamine 80% and xylazine 20%). Blood was drawn from the animal's heart with a syringe under deep anesthesia. Blood samples were then transferred to clean and dry test tubes for serum preparation and centrifuged at room temperature (for blood coagulation) at 3000 rpm for three min after one h serum was removed by sampler and placed in clean vials and stored at -80 °C until hormonal testing. The testes were also separated and after measuring the testes' dimensions, the tissues were placed in fixative (10% formalin solution) for the rest of the experiments. A few days after the end of the experiment, serum levels of testosterone, FSH and LH were measured by Enzyme Linked Immunosorbent Assay (ELISA) kits. The tissue samples were stained by Hematoxylin-Eosin staining (9, 10). All experiments were in accord with the animal care protocol and verified by the local ethics committee.

2.2. Statistical analysis

All data were analyzed by analysis of variance (ANOVA) and $p < 0.05$ was considered as significant. Further analysis by Tukey was done to determine if these groups were different from each other.

3. Results

3.1. Weight change of animals

The weight of all animals was measured both at the start and at the end of the experiment and weight differences were calculated before and after the treatments. No significant changes were observed in any of the groups compared to the control group.

3.2. Biometrics dimensions of the testis

Biometrics dimensions for the testis in all groups were measured and recorded by calibrated ruler and kulis vernier before separating the tissues from the animal body at the end of the experiments. Testis dimensions in the morphine and sulpiride alone groups did not change significantly when compared to the control group (Figure 1). In the pre-injection group, the proportions of testicular morphine-induced seminiferous destructions significantly increased with increasing dose of sulpiride. Also, animals receiving 4 mg/kg of the antagonist had the smallest testicular dimensions

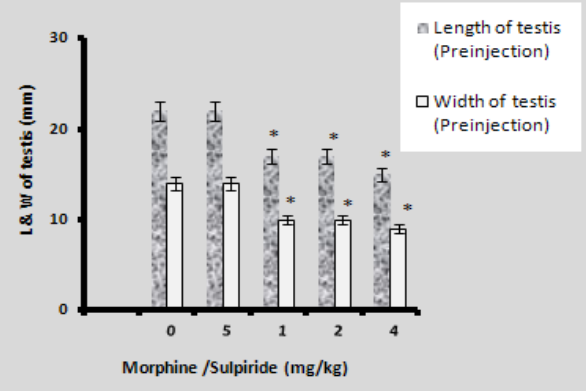

Figure 1. Testicular dimensions in the pre-injection group of sulpiride to morphine.

Reduction of testicular dimensions is evident in pre-injection procedure. * Indicates significant difference ($P<0.05$) versus control group. Zero represents the control group. All data are expressed as mean± SEM.

3.3. Histological features

Histological aspects of the seminiferous tubules and interstitial space in the control group were healthy and normal (Figure 2).

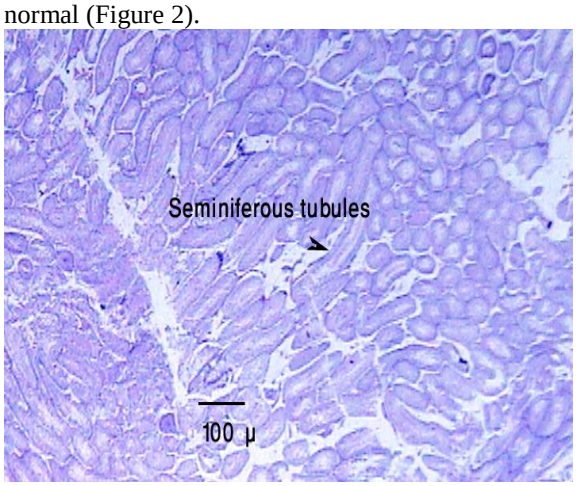

Figure 2. The photomicrographs of the testis of saline group. The seminiferous tubules are evident. Scale bar is 100 µm.

3.3. The effect of morphine on testicular tissue

Morphine was injected i.p. at a dose of 5 mg/kg and the control group received 1 ml/kg of saline i.p. Tissue examinations show that morphine induced testicular damage as compared to the control group (Figure 3).

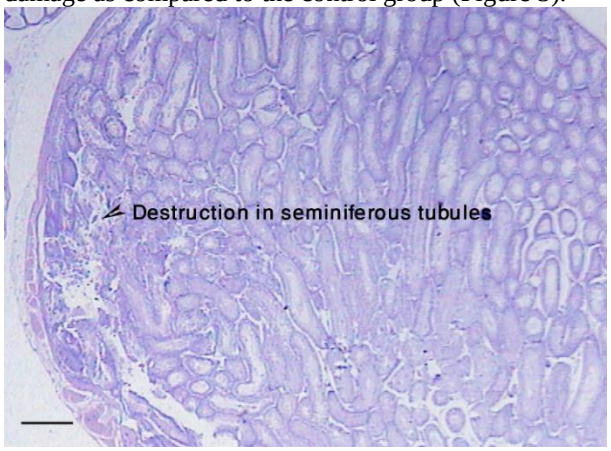

Figure 3. Tissue photomicrographs of the morphine-treated group. Destruction of seminiferous tubules is evident. Scale bar is 100 µm.

3.4. Effect of sulpiride on testicular tissue

Sulpiride alone was injected i.p. to animals at doses of 1, 2 and 4 mg/kg once. According to the observations, the testicular tissue of this group of animals was not significantly different from that of the control group (Figure 4).

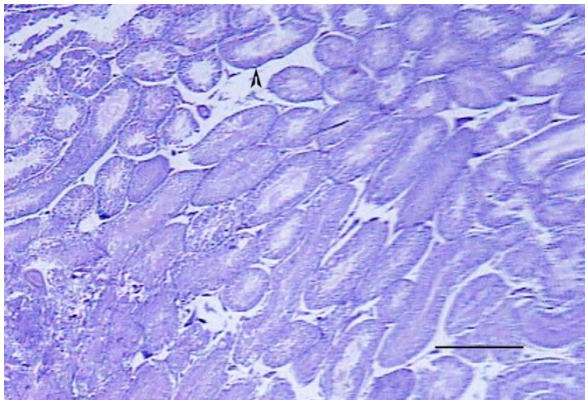

Figure 4. Tissue photomicrographs of the group treated with sulpiride alone, at doses of 2 mg/kg (top image) and 4 mg/kg (bottom image). No significant change was observed in comparison with the control group.

3.5. The effect of pre-injection sulpiride on morphine

Sulpiride was injected i.p. at doses of 1, 2 and 4 mg/kg before pre-injection with 5 mg/kg of morphine (20 min interval). Seminiferous tubules were destroyed in this group, which increased with increasing dose of sulpiride (Figure 5).

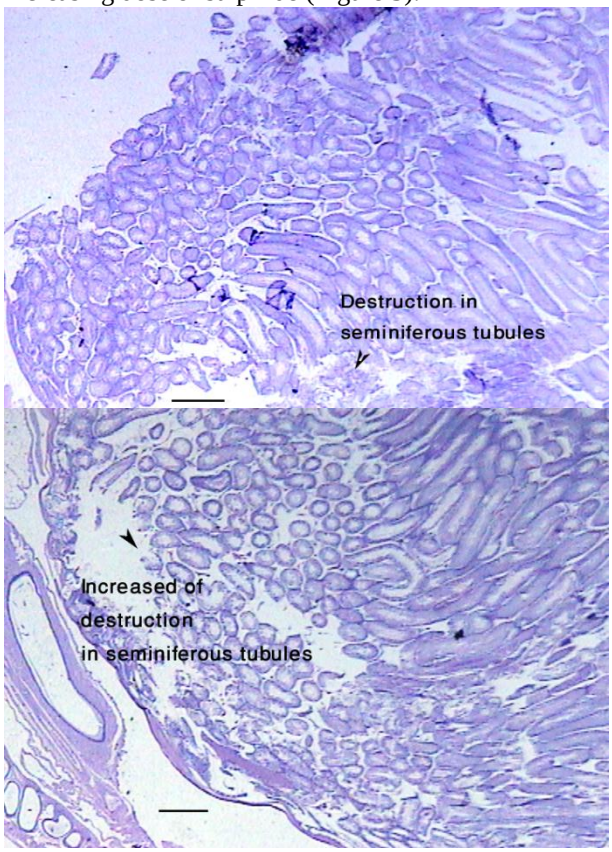

Figure 5. Tissue photomicrographs of the sulpiride-treated group 1 mg/kg (top image) and 4 mg/kg (bottom image) compared to morphine (5 mg/kg). Increased degradation of the seminiferous tubules is evident with increasing doses of sulpiride.

3.6. Results of serum biochemical tests

Serum levels of testosterone, LH and FSH were measured in all groups.

3.6.1. Serum level of testosterone

In the morphine-treated group alone, the serum level of testosterone was significantly decreased. In the sulpiride-treated group, at higher doses, serum levels of this hormone were significantly altered. Following the Tukey test, it was found out that at higher doses of sulpiride, the serum testosterone level decreased. So, this effect was dose-dependent and the level of testosterone decreased as the dose increased. In the pre-injection group of sulpiride prior to morphine, the serum testosterone level significantly increased with increasing dose of sulpiride so that the highest serum level of this hormone was obtained in the sulpiride maximum dose. According to the results, the serum level of testosterone dose-dependently increased in this group (Figure 6).

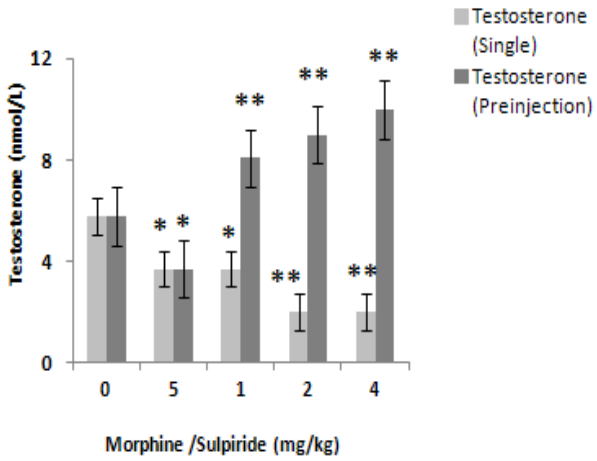

Figure 6. Serum levels of testosterone in all groups. Morphine (5 mg/kg) and sulpiride (1, and 2, and 4 mg/kg) were i.p. injected alone or with morphine (5 mg/kg). Serum testosterone depletion is evident in single injections and a significant increase in pre-injection is achieved. Zero represents the control group. * Indicates significant difference (P <0.05 (between the treated groups and control group). ** indicates significant difference P <0.01 (between the treated groups and control group). All data are expressed as mean ± SEM.

3.6.2. Serum level of LH or FSH

Data were analyzed by ANOVA. According to the figures below, no significant changes were observed in the levels of LH or FSH in either group (P> 0.05) (Figure 7).

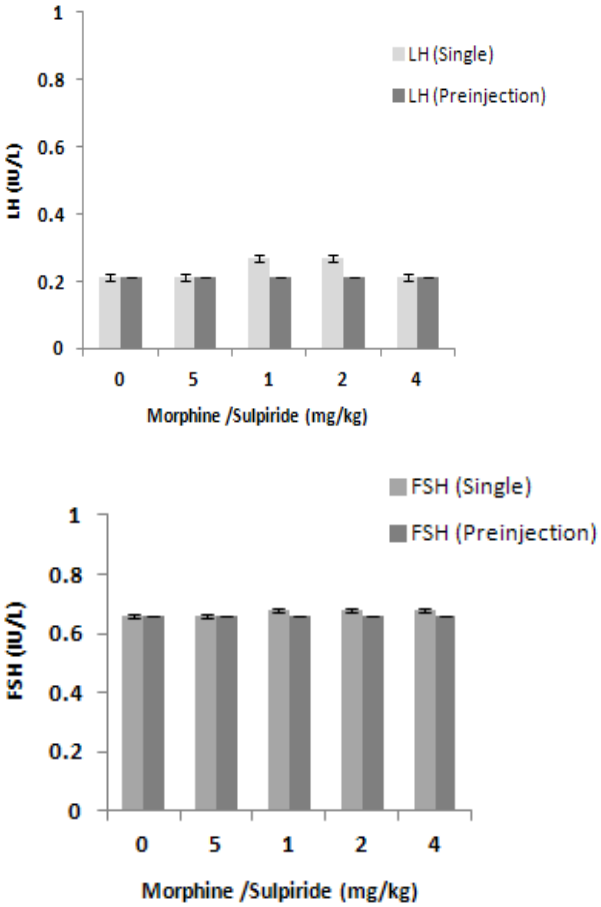

Figure 7. Serum levels of LH or FSH in all groups. Morphine (5 mg/kg) and sulpiride (1, and 2, and 4 mg/kg) were i.p. injected alone or with morphine. Serum values of LH or FSH did not change significantly (P> 0.05). Zero represents the control group. All data are expressed as mean ± SEM

4. Discussion

We aimed to investigate the interaction effect of blocking of D2 dopamine receptor by sulpiride on morphine-induced infertility. Dopamine is one of the basic catecholamines in the biosynthesis pathway of other catecholamines, especially norepinephrine which is centered in the central nervous system (CNS), and epinephrine which is secreted peripherally as a hormone (from endocrine regions such as the central part of the adrenal gland). Although an important part of mood disorders is related to this factor, it is also implicated in male infertility because of its direct effect on the reward axis and the motivational-emotional efficacy of this agent (5).

Endogenous opioid peptides are small molecules that are naturally produced in the CNS and in various glands such as the pituitary and adrenal glands. Today we know that endogenous opioids are also produced in the testicles (11). Exogenous opioids in the brain bind to the same receptors as eendogenous opioids. These

peptides, as neurotransmitters, are involved in regulating physiological processes such as the release of hormones, neurotransmitter release, nutrition, gastrointestinal motility, respiratory activity, sexual behavior and pituitary function. These peptides include enkephalins, and endorphins, and dynorphins (12).

According to the evidence, low dopamine concentrations (<10 μM) increase viability, tyrosine phosphorylation, and sperm motility by activation of the D2 receptor. High concentrations of dopamine (1 mM) decrease tyrosine phosphorylation and sperm motility (6).

GnRH neurons are regulated by dopaminergic agents, augmented serotonin, noradrenergic and endorphin. In particular, GnRH neurons are closely related to dopamine neurons in the hypothalamic aruate nucleus. Dopamine inhibits LH secretion by reducing GnRH secretion and also by directly affecting gonadotrophs.

According to the results of biometrics, testicular dimensions decreased only in the sulpiride-treated groups before morphine as compared to the control group. Injection of sulpiride at a dose of 4 mg/kg as compared to control group had most significant effect.

According to the results of histological studies, the destruction of seminiferous tubules in rats receiving morphine (5 mg/kg) was significantly higher than in the control group. Also, in the sulpiride-treated (1-4 mg/kg) groups treated with morphine, tubule destruction was increased by increasing the dose of sulpiride. However, no significant change was observed in tubules with single dose of sulpiride (1-4 mg/kg).

Serum biochemical tests were performed on three hormones FSH, LH and testosterone. FSH and LH values were not significantly different from the control group in any of the experimental groups. But testosterone levels were significantly different in all groups as compared to the control group. In the groups treated with morphine and sulpiride, the level of testosterone was lower than in the control group dose-dependently. In the sulpiride-treated group, only with increasing doses of the drug did the testosterone decrease more noticeably. But in the pre-injection group of sulpiride rather than morphine, testosterone levels increased so that the dose of testosterone increased as the dose of the drug increased.

Opioids at the hypothalamic level also affect the reproductive axis by decreasing the levels of sex hormone and interfering with GnRH secretion, and consequently by the secondary reduction of FSH and LH secretion. The inhibitory effect of opioids is via the mu receptors in the pre-visual area of the hypothalamus. GnRH production is naturally inhibited by endorphins and is widely stimulated by opioid antagonists. Li and Pelletier found that opioids negatively regulate GnRH mRNA synthesis by *in situ* hybridization, thereby reducing GnRH biosynthesis (13).

Research has also shown that morphine administration has an inhibitory effect on testicular enzymes such as hyaluronidase and acid phosphatase. This inhibition can be accomplished through direct effect on testicular peptide receptors and via the blood levels of gonadotropins. In the male reproductive system, the secretory glands and spermatogenic tubules are androgen-dependent, and the eradication of androgens causes the rapid destruction and apoptosis of spermatogenic tubules (epithelial cells), prostate, seminal vesicles, and other tissues become dependent. The most significant effect of testosterone on the male body is in the target tissues to increase protein production (14). Results from a 2015 study showed that chronic administration of morphine (5 mg/kg) i.p. reduced cell density in the walls of spermatozoon tubules and cells proliferated and irregularly; spermatogonia, primary spermatocytes, spermatids and Leydig were significantly reduced. These results are similar to the results of the present study from the perspective that the degradation of the seminal tubules in the morphine receiving group was only evident.

The mechanism of action of sulpiride is to specifically block D2 dopamine receptors, which disrupts the dopamine control system on lactotrophic cells (prolactin secretor in the anterior pituitary) resulting in hyperprolactinemia (15). Prolactin plays a key role in regulating sexual and reproductive behavior and activity (16). In general, prolactin is the regulator of the hypothalamic-pituitary-testis axis. As mentioned earlier, testosterone synthesis is required for sperm production and supplementation and for enhancing secondary sexual characteristics and normal sexual behavior (17). These actions are controlled by pulsatile GnRH secretion by the hypothalamus as well as LH and FSH secretion from the anterior pituitary. High concentrations of prolactin have inhibitory effects in humans and animals. Hyperprolactinemia reduces GnRH secretion, thereby reducing the pulsatile LH secretion and to a lesser extent FSH (18). Debeljuk and colleagues investigated the effects of acute administration of sulpiride intravenously. They reported elevated serum prolactin levels but no significant changes in serum FSH and LH levels (19). In the present study, serum levels of FSH and LH were not significantly different in the sulpiride-treated groups. Although we have not yet measured serum levels of prolactin, it is likely to have increased (in the future we should assay). Oseko and his colleagues reported similar results. Based on their tests, oral administration of sulpiride increased serum prolactin levels in volunteers, but FSH and LH did not change significantly (20).

In the group receiving sulpiride prior to morphine, testosterone levels increased with increasing dose of sulpiride (dose dependent). This was due to the interaction between the dopamine D2 receptor and the

opioid receptor at the testicular surface. In fact, it can be said that the morphine signaling pathway is different between the two states where the D2 receptors are not blocked or blocked. In other words, the morphine signaling pathway is dependent on dopamine. Although extracellular testosterone levels increased, the tubules of the seminal vesicles were destroyed and testis size decreased. These results may be due to hyperprolactinemia caused by sulpiride pre-injection, and the effect of prolactin on prolactin receptors in Leydig cells (reduced androgen binding proteins count). These effects may also be the result of blocking D2 dopamine receptors on testicular cell surface and eliminating its regulatory effects on spermatogenesis. Another possibility is that the binding pathway of proteins plays a role in dopamine, which, unlike dopamine, does not have the ability to take testosterone. All of these possibilities will need to be scrutinized in the future for clarification

Acknowledgement

We thank Research deputy of Shahed University for support of M. Sc. program.

References

1. Safarinejad MR. Infertility among couples in a population-based study in Iran: prevalence and associated risk factors. International Journal of Andrology 2008;31(3):303-14.
2. Ahmadnia H, Rezayat A, Hoseyni M, Sharifi N, Khajedalooee M, et al. Short-Period Influence of chronic morphine exposure on serum levels of sexual hormones and spermatogenesis in rats. Nephro-Urology Monthly 2016;8(4):e38052.
3. Zegers-Hochschild F, Adamson GD, de Mouzon J, Ishihara O, Mansour R, Nygren K et al. International Committee for Monitoring Assisted Reproductive Technology (ICMART) and the World Health Organization (WHO) revised glossary of ART terminology. Fertility and Sterility 2009;92(5):1520-4.
4. Boivin J, Bunting L, Collins JA, Nygren KG. International estimates of infertility prevalence and treatment-seeking: potential need and demand for infertility medical care. Human Reproduction 2007;22(6):1506-1512.
5. Semet M, Paci M, Saıas-Magnan J, Metzler-Guillemain C, Boissier R, Lejeune H et al. The impact of drugs on male fertility: a review. Andrology 2017;5:640-663.
6. Ramírez AR, Castro MA, Angulo C, Ramió L, Rivera MM, Torres M et al. The presence and function of dopamine type receptors in bear sperm: a possible role for dopamine in viability, capacitation, and modulation of sperm motility. Biology of Reproduction 2009;80:753-61
7. Urra JA, Villaroel-Espíndola F, Covarrubias AA, Rodríguez-Gil JE, Ramírez-Reveco A et al. Presence and function of dopamine transporter (DAT) in stallion sperm: Dopamine modulates sperm motility and acrosomal integrity. PloS one 2014;9:112834
8. Nasehi M, Piri M, Pournaghshband M, Aghazadeh Amiri M, Ramazankhani O. Interaction between dopamine D2 and NMDA receptors in the dorsal hippocampus of rats in the elevated plus-maze test of anxiety. Scientific Journal of Kurdistan University of Medical Sciences 2010;15:29-39.
9. Alan S. "The Haematoxylins". In: Bancroft J, Alan S, (eds.). The Theory and Practice of Histological Techniques (2nd ed.). Longman Group Limited. 1982 p. 109.
10. Titford M. "The long history of hematoxylin". Biotechnic and Histochemistry 2005;80 (2):73-80.
11. Gerendai I, Shaha C, Gunsalus WS, Bardin CW. The effects of opioid receptor antagonists suggest that testicular opiates regulate Sertoli and Leydig cell function in the neonatal rat. Endocrinology 1986;18: 2039-44.
12. Foley KM. Clinical tolerance to opioids. In: Basbaum AI, Besson JM (eds). Towareds a new Pharmacotherapy of pain. John Wiley & Sons, Ltd., New York 1991; 181-03.
13. Li S, Pelletier G. Opioid regulation of gonadotropinreleasing hormone gene expression in themale rat brain as studied by in situ hybridization. Neuroreport, 1993;4:331-3.
14. Guyton and Hall. Textbook of medical physiology. Twelfth edition 2011.
15. Pandiyan N. Medical drugs impairing fertility. Reproductive Health and the Environment 2007;187-05.
16. Clayton AH. Sexual function and dysfunction in women. Psychiatric Clinics of North America 2003;26, 673-82.
17. Pardridge WM, Gorsky RA, Lippe BM, Green R. Androgens and sexual behavior. Annals of Internal Medicine 1982;96,488-01.
18. Murry FT, Cameron DF, Ketehum C. Return of gonadal function in men with prolactin-secreting pituitary tumors. Journal of Clinical Endocrinology 1984;59,79-85.
19. Debeljuk L, Rozados R, Daskal H, Velez V, Mancini AM, Acute and chronic effects of sulpride on serum prolactin and gonadotropin levels in castrated male rats, Proceedings of the Society for Experimental Biology and Medicine 1975;148(2):550-2.
20. Oseko F, Oka N, Furuya H, Morikawa K. Effects of chronic sulpiride-induced hyperprolactinemia on plasma testosterone and its responses to hCG in normal men. Journal of Andrology 1988; 9(4).