Cellular and Molecular Biology
E-ISSN : 1165-158X / P-ISSN : 0145-5680
www.cellmolbiol.org

Treadmill exercise has healing effects on obesity-induced sexual behavior disorder through kisspeptin and kiss1R expression in male rats

Emine Kacar[1], Ozgur Bulmus[2], Zubeyde Ercan[3], Ilay Buran Kavuran[4], Gokhan Zorlu[6], Fatih Tan[5*], Ihsan Serhatlıoglu[6], Haluk Kelestımur[7]

[1] Firat University, Medicine Faculty, Department of Physiology, Elazig, Turkey
[2] Balikesir University, Faculty of Health Sciences, Department of Physical Therapy and Rehabilitation, Balikesir, Turkey
[3] Firat University, Faculty of Health Sciences, Department of Physical Therapy and Rehabilitation, Elazig, Turkey
[4] Firat University, Faculty of Medicine, Department of Medical Biology, Elazig, Turkey
[5] Osmaniye Korkut Ata University, Vocational School of Health Services, Osmaniye, Turkey
[6] Firat University, Faculty of Medicine, Department of Biophysics Elazig, Turkey
[7] Istanbul Okan University, Faculty of Medicine, Department of Physiology, Istanbul, Turkey

ARTICLE INFO

Original paper

Article history:
Received: July 4, 2022
Accepted: March 13, 2023
Published: March 31, 2023

Keywords:

Obesity, sexual behavior, treadmill exercise, Kiss1, Kiss1R

ABSTRACT

The basic objective of this study was to examine the possible effects of treadmill exercise on obesity-related sexual behavior disorder in obese male rats and the role of kisspeptin in this effect. The rats were separated from their mothers at the age of 3 weeks, and classified into four groups as Control (C): normal diet-sedentary group, Exercise (E): normal diet-exercise group, Obese (O): high-fat diet-sedentary group, Obese + Exercise (O+E): high-fat diet-exercise grouSexual behavioral testing was conducted in the rats. At the end of the study, brain samples were taken from the animals for gene expression analyses. The treadmill exercise caused a significant increase in the O+E Group compared to the O Group in kisspeptin and kiss1R gene expression and in EF, ML, IL, MF, IF, III, EL, PEI, IR1, MFT, IFT, IRT sexual behavior parameters ($p<0.05$), and a significant decrease in ML, IL, III, EL sexual behavior parameters ($p<0.05$). Treadmill exercise caused a significant decrease in EF, ML, IL, MF, IF, III, EL, PEI, IR1, MFT, IFT, IRT sexual behavior parameters and kisspeptin and kiss1R gene expression in the hypothalamus, hippocampus, prefrontal cortex and corpus striatum in E Group compared to C Group ($p<0.05$), and a significant increase in ML, IL, III, EL sexual behavior parameters ($p<0.05$). Based on this effect, we believe that it is caused by an increase in kisspeptin and kiss1R expression in the hypothalamus, hippocampus, prefrontal cortex and corpus striatum. In conclusion, treadmill exercise-induced kisspeptin secretion may increase GnRH secretion and cause hypothalamo-pituitary gonadal axis activation and ameliorative effect on deteriorated sexual function.

Doi: http://dx.doi.org/10.14715/cmb/2023.69.3.2

Introduction

Obesity is defined as the excessive accumulation of fat in the body, mainly because of the deterioration in the balance between energy intake and spending (1). Deterioration in the balance between energy intake and consumption is based on over-consumption of calorie-rich foods, physical inactivity and a sedentary lifestyle (2). Obesity is a multisystemic health problem that causes the development of many diseases such as diabetes, hypertension, cardiovascular diseases, including infertility and reproductive system pathologies (3, 4). The dramatic increase in the probability especially in young adults of reproductive age also highlighted the problem of infertility, which is one of the major complications of obesity (5, 6). Obesity is one of the first reasons when considering the causes of male infertility in the last two decades (7). For these reasons, new approaches were developed in recent years to treat obesity-related infertility. Although there are many treatment approaches for infertility treatment, studies are showing that physical exercise, which is one of them, has positive effects, especially in male infertile individuals. Although there are also several other studies arguing against this, the conviction that exercise has positive effects, especially in the treatment of male infertility, is more dominant (8, 9). Although the reproductive system has a complex physio-pathological mechanism, there are still many unknown aspects; however, efforts to illuminate dark spots are continuing in a fast manner. Also, while trying to illuminate the physiological mechanisms of the reproductive system in recent years, kisspeptin came to the forefront as an important way of revealing the physiological mechanisms of the reproductive system with the discovery of the hormone. Kisspeptin is a peptide with key roles in the regulation of reproduction by binding to G protein-bound receptor GPR54, which is also known as Kiss 1-Receptor (10). Kisspeptin was first discovered in 1996, and its effects on the reproductive system were discovered in 2003 (11, 12). The main physiological function of Kisspeptin is to provide GnRH release from hypothalamic GnRH neu-

* Corresponding author. Email: fatihtan@osmaniye.edu.tr

rons. This is how it shows its effect on the reproductive system (13, 14).

Considering the current data, obesity-related infertility is a major health problem. Considering that the main reason for obesity is the imbalance between energy intake and spending, we believe that exercise, which has had positive effects in the treatment of infertility in previous studies, may be an effective treatment method in the treatment of obesity-related infertility. For this, we aimed to examine the possible change in many parameters of sexual behavior, which are almost impossible to measure in human beings by applying a High Fat Diet (HFD), by applying treadmill exercise in male rats with obesity. Also, to illuminate the physio-pathological mechanism under the possible healing role of treadmill exercise on infertility because of obesity in male rats, to detect the change in Gpr54 gene expression, kisspeptin and Kisspeptin receptor in the brain regions associated with reproduction in rats with Real Time Polymerase Chain Reaction (RT-PCR) method. As a result of the present study, we aimed to reveal the possible healing role of treadmill exercise, and the role of kisspeptin in obesity-induced sexual behavior impairments.

Materials and Methods

Animals and Experimental Design

Prepubertal Sprague-Dawley male rats, aged 21 days and weighing 40±2 g, were obtained from the University of Firat Experimental Research Unit (Elazig, Turkey). The experimental protocols were approved by Firat University Ethical Committee, and the rats were treated in accordance with the national and international laws and policies on the care and use of laboratory animals. The animals were weaned at day 21 postpartum and were housed under a reversed light/darkness schedule (12 h light: 12 h darkness from 0700 h), at constant temperature (21±1°C) and humidity (55±5%) with free access to pelleted food and tap water.

After the rats were separated from their mothers at the age of 3 weeks, they were randomly divided into two groups with different diets: (i) normal rat laboratory chow, ND (total 2.65 kcal g− 1 carbohydrate 67.3 %, fat 4.33 % and protein 24.17 %; Korkutelim Yem Gıda San. Tic. AŞ., Korkuteli, Antalya, Turkey); (ii) obesity-inducing high-fat diet, HFD (total 5.24 kcal g− 1— carbohydrate 26%, fat 35% and protein 26%; diet #D12492, Research Diet, New Brunswick, NJ, USA).

The rats were kept undisturbed until they reached 16 weeks of age, and only the HFD rats that were obese (according to the Lee Index) after 16 weeks of feeding were selected. Because obesity generally starts in childhood and continues into adulthood, rats in the prepubertal period were included in our study. The HFD model reliably produced obesity in rats, as indicated by Lee index (15), which we used to determine obesity in the rats. The Lee index for assessing obesity in rats is similar to BMI in humans. It was defined by Lee in 1929 (16) as the cube root of body weight (g) divided by the naso–anal length (cm) and multiplied by 1000. Values greater than 310 were considered an indicator of obesity (17). Thereafter, each group (ND and HFD) was further randomly sub-divided into two groups, one control (C) and one exposed to exercise (E). Thus, all the rats were classified into four groups which were the control (C): normal diet-sedentary group, exercise (E): normal diet-exercise group, obese (O): high-fat diet-sedentary group, obese + exercise (O+E): high-fat diet-exercise grouThese four groups were bred for nineteen weeks. The sample size for all the experiments was of n = 10 for each grouThe body weights (individual rats) of all animals were measured weekly over the course of the experiment. The sexual behavior test was performed on the 20th day after starting the exercise.

Exercise Protocol

After obesity was induced by a 16-week high-fat diet, treadmill exercise was performed for 6 weeks. Rats in exercise groups were subjected to moderate-intensity exercise on a motor-driven treadmill (May TME 0805 Treadmill Exerciser) continuously for a period of 6 weeks between 09.00 am and 10.00 am. The E rats exposed to ND and HFD ran at a speed of 15 m/min and 25 m/min at the beginning and end, respectively. The speed was periodically increased throughout the experiment. The duration of exercise was also periodically increased from 15 min at the beginning to 50 min at the end of the experiment. We preferred to increase the speed and duration of the exercise periodically in order that the exercise that continues at the same level will cause habitation. In order to encourage the rats to run continuously, the metal bar grid at the beginning of the running lanes constantly delivered a mild foot shock (intensity=0.5 mA).

Sexual Behavior Test (SBT)

All sexual behavioral tests were performed during the dark phase between 13:00 h and 16.00 h in a room with a night vision camera system. Each rat was placed in a rectangular observation cage (40x50x65) made from Plexiglas. The rat was allowed to habituate for 15 min and then a receptive female was placed in the arena. Each test lasted 30 min. Rats that did not ejaculate within 30 min were excluded from the analysis. Video tape-recordings were later replayed and analyzed in slow motion. The following parameters for the first two ejaculation series were measured: (ML) mount latency (time between the introduction of the female into the cage and the first mount); (IL) intromission latency (time between the introduction of the female into the cage and the first intromission); (EL) ejaculation latency (time between the first intromission and ejaculation); (EF) ejaculation frequency (number of ejaculations in each copulatory series); (PEI) post-ejaculatory interval (interval between each ejaculation and the next copulatory act); (MF) mount frequency (number of mounts prior to the first ejaculation); (IF) intromission frequency (number of intromissions prior to the first ejaculation); (III) interval between intromissions in first mating series; (MFT) total mount frequency for the 30-minute test; (IFT) total intromission frequency for the 30-minute test; IR1 (IF1 / IF1 + MF1); (IRT) copulatory efficiency (intromission frequency/intromission + mount frequency).

Gene Expression Analysis by Real-Time-Polymerase Chain Reaction (RT-PCR)

At the end of the experiment, the brains of decapitated animals were quickly removed. The brain parts such as the hypothalamus, hippocampus, corpus striatum and prefrontal cortex areas were isolated and frozen in liquid nitrogen. Brain sections were stored at -80°C until analyzed. Gene

Table 1. List of genes analyzed by RT-PCR method.

Symbol	Genes
Kiss1	Kisspeptin
Kiss1R	Gpr54, Kisspeptin receptor

expression analysis of neuropeptides and neurotransmitters and their receptors related to obesity, exercise and behavioral changes in the brain regions was performed using the Real Time Polymerase Chain Reaction (RT-PCR) method. The $2^{-\Delta\Delta CT}$ method was used to calculate differences between gene expressions. Gene expression levels were determined using the Applied Biosystems 7500 Real Time PCR system (Applied Biosystem, Foster City, CA, USA). GAPDH was used as a control gene (housekeeping) in the study. The heat conditions were set to 1 time at 95°C for 15 min and 40 times at 95°C for 15 s, at 60°C for 30 s, and at 72°C for 30 s. The genes analyzed are shown in Table 1.

Statistical Analyses

Values were expressed as mean±S.E.M. One-way analysis of variance (ANOVA) and Post-Hoc Tukey test were used to assess the data and differences between groups. For all analyses, p<0.05 was considered statistically significant.

Results

Effects of exercise and obesity on the sexual behavior test (SBT).

As seen in Table 2, obesity decreased ejaculation frequency (EF) (0.1±0.1) significantly compared to all other groups (p<0.001). The average numbers of ejaculation were statistically similar in the C, E and O+E groups, being 2.0±0.0, 2.4±0.17 and 1.5±0.4, respectively. The average values of ML and IL significantly were higher in the O group compared to all other groups. ML and IL did not show significant differences between the C, E and O+E groups. The obese rats exposed to exercise had higher mount frequency than the E group while they had higher IF than all the groups. The duration of the intervals between intromissions (III) in the C, E and O+E groups was lower than the O (p<0.01) grouIFT; for the 30-min test were higher in the O+E group than the C (p<0.01) and the E and O groups (p<0.01).

Gene Expression Analysis by RT-PCR Method

The effects of high fat diet and exercise on gene expression in different brain regions are shown in Figure 1 as fold increase. In the gene expression analysis, kiss 1 and kiss 1R (kisspeptin and kisspeptin receptor), levels were reduced in all brain regions in O group compared to con-

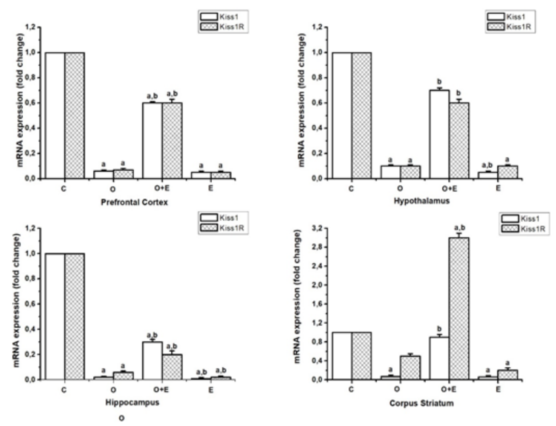

Figure 1. Demonstration of Gene Expression by RT-PCR as a fold increase in brain regions.

Table 2. Sexual Behavior Test Results.

Parameter	Control	Exercise	Obese	Obese+Exercise
EF (#)	2±0^{a*}	2.4±0.17^{a*}	0.1±0.1	1.5±0.4^{a*}
ML (s)	38.4±10.1^{a**}	59.8±13.6^{a**}	483±166.3	65.1±43.1^{a**}
IL (s)	43.8±11.7^{a**}	31.3±7.7^{a**}	257±89.1	39.5±18.06^{a**}
MF (#)	8.5±1.01	6.6±1.9^{b*}	8.8±2.6	15.2±2.6
IF (#)	13.5±1.1^{b*}	9.5±1.7^{b*}	11.3±4.1^{b*}	28.3±6.3
III (s)	23.1±0.9^{a**}	25.2±2.2^{a**}	78.9±22.6	18.8±4.9^{a**}
EL (s)	619±51.6^{a**}	456±67.3^{a**}	1460	845.7±172.6^{a**}
PEI (s)	416.1±23^{a**}	402.8±18.9^{a**}	0	258.6±66.5$^{a**, c*}$
IR1	0.61±0.02^{a*}	0.61±0.04	0.39±0.1	0.62±0.04^{a*}
MFT (#)	15.1±1.7	16.3±3.9	8.87±2.6	24.1±3.6^{a*}
IFT (#)	23.3±1.2^{b*}	19±1.9^{b*}	11.3±4.1^{b*}	41.9±5.8
IRT	0.61±0.02^{a*}	0.56±0.03	0.39±0.1	0.63±0.04^{a*}

Data are presented as mean ± S.E.M. ML = Mount latency. IL = intromission latency. MF = mount frequency. IF = intromission frequency. III = interval between intromissions in first mating series. EL = ejaculation latency. PEI = post ejaculatory interval. EF = ejaculation frequency. MFT = total mount frequency for the 30 minute test. IFT = total intromission frequency for the 30 minute test. IR1: IF1 / IF1 + MF1. IRT: IFT / (IFT + MFT).

a Compared with obese grou

b Compared with obese+exercise group.

c Compared with control group (* p<0,05. ** p<0,001).

trol and O+E grou.

Discussion

There are many studies conducted on the effects of obesity and exercise on reproductive performance in humans and animals. However, so far, there are no studies on the effects of exercise on obesity-related sexual behavior disorder induced by HFD in rats. For this reason, our study is one of the first studies in this field in this sense.

According to the results obtained in this study, treadmill exercise improves sexual behavior disorder in male rats with obesity-induced with HFD. Also, the increase of kisspeptin and Gpr54 expression in the brain regions associated with reproduction in male rats that did exercise suggests that kisspeptin and treadmill exercise plays roles in the healing of sexual behavior disorder in obese male rats.

Approximately 15% of couples are infertile worldwide. Male infertility is the cause of primary infertility in approximately 20% of couples, and 30-40% of them is among the reasons that contribute (18, 19). When the causes of male infertility are considered in the last two decades, it is seen that obesity is one of the most important causes (20, 21). For this reason, efforts are spent develop new treatment approaches in obesity-related infertility, like in other pathologies related to obesity. When the major causes of obesity were examined, there are deterioration of the balance between energy intake and consumption, over-consumption of calorie-rich foods, physical inactivity and sedentary life (2). For this reason, it is possible that exercise may be a treatment option to treat infertility because of obesity. Studies that investigated the effect of exercise on obesity-related infertility are limited so far (22). In one previous study, after healthy eating in 43 men with unexplained infertility and weight loss with daily exercise for 14 weeks, it was found that weight loss was associated with increases in total sperm counts (193 million; 95% CI 45 to 341) and morphology (4%; 95% CI 1 to 7%) (23). Also, a recent prospective study (n = 23) evaluated the effects of excessive weight loss on sperm quality in six months following bariatric surgery (24). Although it was not significant in sperm counts and mobility, a positive trend was found with a significant increase in semen volume (difference: +0.6 mL, p = 0.04) and vitality (difference: +10%, p = 0.03). These and similar studies did not evaluate the effectiveness of exercise in obesity-related infertility and sexual behavior disorders beyond examining the change in sperm morphology, which is the only parameter that can only be measured in this respect.

The limited data obtained here are not sufficient, and precise data and need further examination. It is almost impossible to determine the change in sexual behavior parameters (EF, ML, IL, MF, IF, III, EL, PEI, IR1, MFT, IFT and IRT, etc.) that are the most important measures in terms of reproductive performance. Studies are carried out in this field only as surveys because of limited reliability and correctness. In this sense, our study is the first one that examines the change in sexual behaviors because of obesity in male rats and the effectiveness of exercise on this change. When our findings were examined, it was found that EF, MF, IF, PEI, IR1, MFT, IFT, IRT (Table 2), which show positive sexual performance (25), were lower at statistically significant levels in obese male rats ($p<0.05$) when compared to the control group, and ML, IL, III, EL (Table 2), which show negative sexual behavior (25), were higher in obese animals statistically ($p<0.05$). However, obese male rats that did exercises had close values to the Control Group, in which the same parameters were found to improve when compared to those of the obese. EF, MF, IF, PEI, IR1, MFT, IFT, IRT parameters (Table 2), which are positive sexual behavior indicators in the group that did exercise were lower ($p<0.05$) when compared to the controls, and ML, IL, III, EL (Table 2) values were higher ($p<0.05$) when compared to controls. Although the current finding is that exercise improves obesity-related sexual behavior disorder in male rats, it can be seen to be quite confusing to see that exercise negatively affects sexual behavior parameters in normal-weight male rats at the same level. However, a similar study showed that involuntary exercise negatively affects semen parameters in normal-weighted males (8). In this respect, it supports the data obtained by us. Also, the positive change in the sexual behavior parameters, there was a correlated with the increase in kisspeptin levels.

The expression of Kisspeptin and its receptor, (Figure 1) in the hippocampus, prefrontal cortex, corpus striatum, and hypothalamus, which are brain regions associated with reproduction and behavior, were found to be low ($p<0.05$) in obese rats compared to controls, high and close to controls ($p<0.05$) in the obese + exercise group; and in contrast, it was low ($p<0.05$) compared to controls in the group who did exercises only. It was seen that the EF, MF, IF, PEI, IR1, MFT, IFT, IRT, which are positive sexual behavior parameters, values (Table 2) and ML, IL, III, EL, negative sexual behavior parameters, (Table 2), were directly proportional to the changes between the groups. In light of these findings, it is concluded that the increase in kisspeptin in brain regions may be associated with positive sexual behavior. In other words, we are of the opinion that treadmill exercises caused increases in kisspeptin levels and also plays role in the healing effect of sexual behavior disorder in obese rats. In our study, it was found that exercise negatively affects sexual behavior parameters, decreasing kisspeptin and kisspeptin 1R expression in the associated brain regions, which supports our findings that support with Arisha and collaborates (26), a study which found that forced swimming in normal weight male rats reduced hypothalamic kisspeptin and kiss1R expression, lowering sex steroids in these rats. It also reported that forced exercise reduced kisspeptin and kisspeptin 1R expression in normal-weight male rats, which resulted in a decrease in sex steroid levels. The findings of this study support our findings.

In a conclusion, based on the findings obtained in our study, treadmill exercise improves the sexual behavior disorder caused by obesity in obese male rats. In the light of our findings, we believe that Treadmil exercise provides the ameliorating effect of obesity-related sexual behavior disorder by increasing the expression of kisspeptin and kiss1R in the reproductive brain regions of the hypothalamus, hippocampus, prefrontal cortex and corpus striatum. We think that increased kisspeptin secretion may increase GnRH secretion and cause hypothalamo-pituitary gonadal axis activation and ameliorative effect on deteriorated sexual function.

Fundings

This study was supported by Turkish Scientific Technical

Research Organization (TUBITAK Project No: 114S179).

Data availability
The data that support the findings of this study are available from the corresponding author upon reasonable request.

Credit author statement
E.K., I.S. and H.K. designed and supervised the study. O.B., Z.E. and I.B.K. conducted all animal experiments. I.S., O.B. and Z.E. conducted all hormone measurements. F.T. and G.Z. contributed to data analysis. E.K. and H.K. wrote the manuscript, and all authors approved the final manuscript.

Declaration of competing interest
The authors declare there is no conflict of interest with regard todata presented in this article.

Abbreviation List
HFD: High fat diet; **GPR54:** G protein-bound receptor; **RT-PCR:** Real Time Polymerase Chain Reaction
C: Control group; **E:** Exercise group; **O:** Obese group; **O+E:** Obese+exercise group; **ML:** Mount latency
IL: Intromission latency; **EL:** Ejaculation latency; **EF:** Ejaculation frequency; **PEI:** Post-ejaculatory interval; **MF:** Mount frequency; **IF:** Intromission frequency; **MFT:** Total mount frequency; **IFT:** Total intromission frequency; **GADPH:** Gliseraldehid 3-Fosfat Dehidrogenaz; **Kiss 1:** Kisspeptin; **Kiss 1R:** Kisspeptin receptor.

References

1. Gatta-Cherifi, B. and D. Cota, New insights on the role of the endocannabinoid system in the regulation of energy balance. Int J Obes (Lond), 2016; 40(2): 210-9.
2. von Deneen, K.M. and Y. Liu, Obesity as an addiction: Why do the obese eat more? Maturitas, 2011; 68(4): 342-345.
3. Weiss, A.L., A. Mooney, and J.Gonzalvo, Bariatric surgery: the future of obesity management in adolescents. Adv Pediatr, 2017; 64(1): 269-283.
4. Rajan, T. and V. Menon, Psychiatric disorders and obesity: a review of association studies. J Postgrad Med, 2017; 63(3): 182.
5. Memedi, R., Tasic V, Nikolic E, Jancevska A, Gucev Z., Obesity in childhood and adolescence genetic factors. Pril (Makedon Akad Nauk Umet Odd Med Nauki), 2013; 34(2): 85-89.
6. Wang, Y. and T. Lobstein, Worldwide trends in childhood overweight and obesity. Int J Pediatr Obes, 2006; 1(1): 11-25.
7. Leisegang, K. and S. Dutta, Do lifestyle practices impede male fertility? Andrologia, 2021; 53(1): e13595.
8. Ibañez-Perez J, Santos-Zorrozua B, Lopez-Lopez E, Matorras R, Garcia-Orad A., An update on the implication of physical activity on semen quality: a systematic review and meta-analysis. Arch Gynecol Obstet, 2019; 299(4): 901-921.
9. Agarwal A, Parekh N, Selvam MKP, Henkel R, Shah R, Homa ST, et al., Male oxidative stress infertility (MOSI): proposed terminology and clinical practice guidelines for management of idiopathic male infertility. World J Mens Health, 2019; 37(3): 296.
10. Yun S, Kim D-K, Furlong M, Hwang J-I, Vaudry H, Seong JY, Does kisspeptin belong to the proposed RF-amide peptide family? Front Endocrinol (Lausanne), 2014; 5: 134.
11. Lee J-H, Miele ME, Hicks DJ, Phillips KK, Trent JM, Weissman BE, et al., KiSS-1, a novel human malignant melanoma metastasis-suppressor gene. JNCI: J Natl Cancer Inst, 1996. 88(23): 1731-1737.
12. Seminara SB, Messager S, Chatzidaki EE, Thresher RR, Acierno Jr JS, Shagoury JK, et al., The GPR54 gene as a regulator of puberty. N Engl J Med, 2003; 349(17): 1614-1627.
13. Gottsch ML, Cunningham MJ, Smith JT, Popa SM, Acohido BV, Crowley WF, et al., A role for kisspeptins in the regulation of gonadotropin secretion in the mouse. Endocrinology, 2004; 145(9): 4073-4077.
14. Han S-K, Gottsch ML, Lee KJ, Popa SM, Smith JT, Jakawich SK, et al., Activation of gonadotropin-releasing hormone neurons by kisspeptin as a neuroendocrine switch for the onset of puberty. J Neurosci, 2005; 25(49): 11349-11356.
15. Bernardis, L.L. and B.D. Patterson, Correlation between 'Lee index' and carcass fat content in weanling and adult female rats with hypothalamic lesions. J Endocrinol, 1968; 40(4): 527-8.
16. Lee, M.O., The function of the air sacs in holopneustic insects. Science, 1929. 69(1786): 334-5.
17. Hariri, N. and L. Thibault, High-fat diet-induced obesity in animal models. Nutr Res Rev, 2010; 23(2): 270-299.
18. Esteves SC, Hamada A, Kondray V, Pitchika A, Agarwal A, et al., What every gynecologist should know about male infertility: an update. Arch Gynecol Obstet, 2012; 286(1): 217-229.
19. Hamada, A., S. C Esteves, and A. Agarwal, The role of contemporary andrology in unraveling the mystery of unexplained male infertility. Open Reprod Sci J, 2011; 3(1).
20. Baskaran S, Agarwal A, Leisegang K, Peter NP, Selvam MKP, Henkel R., An in-depth bibliometric analysis and current perspective on male infertility research. World J Mens Health, 2019.
21. Baskaran, S., Agarwal, A., Panner Selvam, M. K., Finelli, R., Robert, K. A., et al., Tracking research trends and hotspots in sperm DNA fragmentation testing for the evaluation of male infertility: a scientometric analysis. Reprod Biol Endocrinol, 2019; 17(1): 1-13.
22. Best, D., A. Avenell, and S. Bhattacharya, How effective are weight-loss interventions for improving fertility in women and men who are overweight or obese? A systematic review and meta-analysis of the evidence. Hum Reprod Update, 2017; 23(6): 681-705.
23. Håkonsen, L. B., Thulstrup, A. M., Aggerholm, A. S., Olsen, J., Bonde, J. P., Andersen, C. Y., et al., Does weight loss improve semen quality and reproductive hormones? Results from a cohort of severely obese men. Reprod Health, 2011; 8(1): 1-8.
24. Samavat, J., Cantini, G., Lotti, F., Di Franco, A., Tamburrino, L., Degl'Innocenti, S., et al., Massive weight loss obtained by bariatric surgery affects semen quality in morbid male obesity: a preliminary prospective double-armed study. Obes Surg, 2018; 28(1): 69-76.
25. Ågmo, A., Male rat sexual behavior. Brain Res Brain Res Protoc, 1997. 1(2): 203-209.
26. Arisha, A.H. and A. Moustafa, Potential inhibitory effect of swimming exercise on the Kisspeptin–GnRH signaling pathway in male rats. Theriogenology, 2019; 133: 87-96.