The Effect of Aerobic Training on Serum Levels of Adiponectin, Hypothalamic-Pituitary-Gonadal Axis and Sperm Quality in Diabetic Rats

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Purpose: The present study aims to investigate the effects of aerobic training on adiponectin, sex hormones, and sperm parameters in streptozotocin–nicotinamide induced diabetic rats.

Material and Methods: In this experiment, 52 eight-week-old Sprague Dawley rats (200-250 g) were randomly assigned to four groups: healthy control, diabetic control, diabetic with aerobic training and healthy with aerobic training. Diabetes was induced by intraperitoneal injection of nicotinamide and streptozotocin solution. The aerobic training protocol was performed for ten weeks. Finally, blood serum samples were obtained to assess FSH, LH, testosterone, and adiponectin levels.

Results: Results showed an increase in serum adiponectin levels in aerobic training group which led to a significant difference between aerobic training group and diabetic control group. In addition, aerobic training caused significant increase in serum testosterone level and LH in diabetic aerobic training group, so that significant differences were observed between serum testosterone, LH and FSH of diabetic aerobic training group and healthy control group. Sperm parameters in the diabetic aerobic training group including sperm count, motility and viability presented significant differences compared to diabetic control group.

Conclusion: Short term aerobic training can improve serum adiponectin levels and sperm parameters, including sperm count and sperm motility through increasing serum testosterone, LH and FSH levels in type 2 diabetic rats.

Keywords: adiponectin; diabetes mellitus type 2; aerobic training; sex hormones; sperm parameters.

INTRODUCTION

Diabetes mellitus (DM) is one of the greatest threats to current worldwide health. In a study in 2017, the number of diabetics in the world was 451 million which is anticipated to increase to 693 million in 2045[(1)]. DM may influence male reproductive function at multiple levels as a result of its impacts on endocrine control of spermatogenesis, spermatogenesis itself or by damaging penile erection and ejaculation[(2)]. Data from animal studies strongly suggests that DM impairs male fertility[(3)]. On the other hand, fertility is regulated by two gonadotropic hormones, luteinizing hormone (LH) and follicle-stimulating hormone (FSH) which modulate testosterone synthesis in leydig cells and its aromatization to estradiol in sertoli cells, respectively[(4)]. In a study by Aziz et al. in 2018, remarkable differences between the levels of testicular and pituitary hormones in diabetic rats compared with non-diabetic rats were recognized[(5)]. The findings regarding sperm quality in diabetic rats indicate a decrease in sperm motility and sperm count and an increase in sperm abnormalities[(6)]. Adiponectin plays an important role in metabolic disorders such as obesity, type 2 diabetes, coronary heart disease, and metabolic syndrome[(7)]. Discovery of the metabolic adiponectin hormone has been a main development not only in energy balance, but more generally in fields including reproduction, inflammation, and immunology[(8)].

Regarding the relationship between serum level of adiponectin and its possible effect on fertility, a few studies have been conducted. In 2007, Francisca et al. suggested that the pituitary constitutes a relevant place of action for adiponectin and supports a role for adipokines as a connection in the regulation of metabolism, growth, and reproduction[(9)]. In 2008, Olga et al. also indicated that adiponectin and its receptors are expressed in the chicken testis, where they are likely to affect steroidogenesis, spermatogenesis, sertoli cell function as well as spermatozoa motility[(10)].

Exercise training (ET) is believed to be an important element in the treatment strategy for rats with type 2 diabetes[(11)]. Physical exercise increases glucose disposal into contracting muscles, leading to a significant decrease in blood glucose concentration[(12)].

Despite the beneficial effects of physical exercise on different metabolic aspects of diabetic rats, to the best of our knowledge no study has been conducted to study the effects of physical exercise on fertility of diabetic rats. Therefore, it can be noteworthy to investigate the effect of a period of aerobic training on serum levels of adiponectin, sex hormones and sperm parameters in type 2 diabetic rats.

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Table 1. Aerobic training (AT) protocol (14)

Weeks	Day	AT
	1	20 min, 27 m/min
Week1	2	22 min, 27 m/min
	3	24 min, 27 m/min
	4	24 min, 27 m/min
	5	28 min, 27 m/min
	6	30 min, 27 m/min
Week2	1	32 min, 27 m/min
	2	34 min, 27 m/min
	3	36 min, 27 m/min
	4	38 min, 27 m/min
	5	40 min, 27 m/min
	6	42 min, 27 m/min
Week3	1	44 min, 27 m/min
	2	46 min, 27 m/min
	3	48 min, 27 m/min
	4	50 min, 27 m/min
	5	52 min, 27 m/min
	6	54 min, 27 m/min
Week4	1	56 min, 27 m/min
	2	58 min, 27 m/min
	3	60 min, 27 m/min
	4	60 min, 27 m/min
	5	60 min, 27 m/min
	6	60 min, 27 m/min
Week5-10	1-6	60min, 27 m/min to end of 10th week

MATERIALS AND METHODS

Experimental animals and protocols

Fifty four eight-week-old Sprague Dawley rats (200-250g) were housed in cages in groups of controlled temperature (22 ± 2˚c) and light/dark (12/12h) conditions with free access to water and rat chow. All experimental procedures were guided by regulations approved by the Iranian Ministry of Health. The rats were randomly divided to four groups: control (C) (n = 12), diabetic control (DC) (n = 15), diabetic with aerobic training (DAT) (n = 15) and healthy with aerobic training (HAT) (n = 12) groups. The groups were treated according to the experimental protocol for a duration of 60 days. All experiments were operated in the Medical Science University of Arak. The code of ethics is also included in the description (IR.Arakmu.rec.1394.329) in the ethics committee of the research projects of Arak University of Medical Sciences.

Diabetic induction

Diabetes was induced after a 12 hour fast. The rats were injected with nicotineamid (Sigma Chemical Co) dissolved in normal saline at a dose of 120 mg/kg, and after 15 minutes, streptozotocin (STZ, Sigma Chemical Co) dissolved in 0.1 M citrate buffer at a dose of 65 mg/kg was given in a single intraperitoneal injection. 72 hours after injection, animals' blood glucose levels were evaluated. Those animals that had blood glucose levels higher than 250mg/dL were considered diabetic. Blood glucose levels of the rats were being measured by a glucometer after a 12 h fast. Further, the healthy control rats were given intraperitoneal injections of normal saline at a dose of 1cc to be in the same condition as diabetic groups[13].

Aerobic training protocol

The aerobic exercise was performed on a rodent motor-driven treadmill at a 0˚ slope. The rats exercised for 5 d/w for 10 weeks. Training blocks consist of 3 phases of familiarization, overload, and finally preservation and stabilization of exercise intensity. In the familiarization phase (first week), the rats walked on treadmill at a speed of 8m/min for 10-15 min every day. In the overload phase (second to fourth weeks), the rats initially ran on treadmill at a speed of 27 m/min for 20 min, and then during 3 weeks the time of exercise increased (2min in each session) gradually until reached 60 minutes. Finally, in the preservation and stabilization stage of exercise intensity, the rats did the aerobic exercise for 7 weeks with a speed of 27m/min for 60 min (**Table 1**). Each exercise session began with 5min of warm up (16m/min) and 5min was allocated to cooling down (16m/min and gradual decrease of intensity to the least amount)[14].

Procedure

Twenty four hours after the last exercise session, all of the rats were anaesthetized by the injection of chloroform and sacrificed. Blood samples were collected by cardiac puncture (5cc) and centrifuged at 3500rpm for 10 min and the serum samples were stored at -70˚c for future analysis. Serum levels of testosterone, LH, FSH, adiponectin and insulin were assayed using various kits according to their manufacturer's instructions. Testosterone (Rat ELISA Kit, Eastbiopharm Cat. No Ck-E90243, China, sensitivity: 0.25nmol/L, Assay range: 0.5-100nmol/L), LH (Rat ELISA Kit, Eastbiopharm Cat. No Ck-E90904, China, sensitivity: 0.11mIU/L, Assay range: 0.2-60mIU/L), FSH (Rat ELISA Kit, Eastbiopharm Cat. No Ck-E30597, China, sensitivity: 0.12mIU/L, Assay range: 0.2-60mIU/L), Adiponectin (Rat ELISA Kit, Eastbiopharm Cat. No Ck-E30584, China, sensitivity: 0.16mg/L, Assay range: 0.2-60mg/L) and insulin (Rat ELISA Kit, Eastbiopharm Cat. No Ck-E30620, China, sensitivity: 0.5mIU/L, Assay range: 0.1-40 mIU/L).

Sperm count

The excised left testis was weighed and the dissected epididymis was transferred into 5cc (DMEM) medium and cut into small slices in order to swim out the sperm into the medium. After 10 min of diffusion in 27˚c temperature, 1ml of the solution was diluted with 9ml

Table 2. Body and left testis weight (Means±SD)

Groups Body	Weight (g)		Fasting blood glucose (mg/dl)		Testis Weight left (g)
	Pre test	Post test	Pre test	Post test	
HC	242.9(± 21)	280.3 (± 35)	88.4 (±10)	100.2 (±12)	1.58 (± 0.19)
DC	232.6 (± 36)	253.2 (± 46)	299.3 (±46)[a]	366.4 (±102)[a]	1.31 (± 0.25)[a]
DAT	238.7 (± 20)	227.4 (± 38)[a]	354.2 (±86)[a]	188.8 (±115)b	1.34 (± .32)
HAT	248.7 (± 19)	250.1 (± 25)[a]	85.6 (±8)[c]	76.1 (±4)[b]	1.57 (± .17)[b]

[a]. The significant difference with healthy control group ($p < 0.05$). b. The significant difference with diabetic control group ($p < 0.05$). c. The significant difference with diabetic aerobic training group ($p < 0.05$).

Abbreviations: HC, healthy control group; DC, diabetic control group; DAT, diabetic aerobic training group; HAT, healthy aerobic training group.

Table 3. Epididymal sperm number, sperm motility, sperm viability, and sperm morphology in studied groups.

Groups	Sperm count(10^6)	Sperm Viability (%)	Sperm Motility (%)	Sperm Morphology (%)
HC	39.3 (±13)	77.5 (±4.6)	60.8 (±6.5)	95.4 (±1.3)
DC	11.75 (±5.7) [a]	29.78 (±16.2) [a]	32.5 (±1.1) [a]	85.25 (±7.5) [a]
DAT	26 (±13.2) [ab]	41.7 (±7.2) [ab]	40 (±6.5) [a]	88 (±8.8)
HAT	46.2 (±3.3) [bc]	87.8 (±2.9) [abc]	66.1 (±4.1)[abc]	90.4 (±9.1)[bc]

[a]. The significant difference with healthy control group ($p < .05$). b. The significant difference with diabetic control group ($p < .05$). [c]. The significant difference with diabetic aerobic training group ($p < .05$).
Abbreviations: HC, healthy control group; DC, diabetic control group; DAT, diabetic aerobic training group; HAT, healthy aerobic training group.

formaldehyde fixative. The diluted solution was transferred into each chamber of neubauer hemocytometer and sperm heads were manually counted under a microscope. Sperm count was carried out according to WHO guidelines and data were expressed as the number of sperm per ml[15].

Sperm motility

Measurement of sperm motility was performed according to WHO protocol. 10µl of the sperm suspension was located on a microscope slide and covered. A minimum of five microscope fields were investigated to evaluate sperm motility on at least 200 sperm for each animal, then the percentage of sperm motility was computed[15].

Sperm viability

Eosin-nigrosin staining was used to evaluate sperm viability according to WHO protocol. In this protocol, eosin (1%, Merk, Germany) and nigrosin (10%, Merk, Germany) were prepared in distilled water. At first, one volume of sperm suspension was blended with two volume of 1% eosin, then after 30 seconds an equal volume of nigrosin was added to this mixture. Finally, thin smears were assembled and observed under a light microscope with a magnification of 100X and the ratio of the live sperms percentage in different groups was computed. In this method, viable sperms appeared white while nonviable sperms stained purple[15].

Sperm morphology

Before morphologic investigation of the sperm of each group, smears prepared from sperm suspension stained by the way of papanicolao and then were air dried and utilized according to WHO. In each sample, 100 sperms with a magnification of 100X were investigated and existing abnormalities were reported as a percentage[15].

Statistical analysis

A Shapiro-Wilk test was applied to determine the normality of distribution of measures which were found to be normally distributed. Then a Leven test indicated that the variances were homogeneous. A one-way analysis of variance (ANOVA) and post hoc test (Tukey) was performed to determine differences among the groups. Data were expressed as means ± SD and significance was set at the alpha level $p < .05$. Correlation between variables was also determined by Pearson correlation coefficient.

RESULTS

During the implementation of the training, 3 rats were excluded from the study because of diabetic complications in the diabetic control group, 2 rats died during the training protocol and 1 rat was removed from the diabetic aerobic training due to failure to perform the training protocol.

Fasting blood glucose, Body weight, and left testis weight

For each animal, body weight was recorded at the beginning and end of a 70-day period.
Comparison of weight factor in posttest showed a significant difference between healthy control group with diabetic training group ($P = .012$) and healthy training group ($P = .032$) (**Table 2**).
Also, there was significant difference in the mean left testis weight of rats in the healthy control group compared to diabetic control group ($P = .021$) (**Table 2**). There was no significant difference in fasting blood glucose level of diabetic control group and diabetic aerobic training group in the beginning of the period ($P = .311$), but after 10 weeks of aerobic training there was a significant decrease in fasting blood glucose of diabetic aerobic training group compared to diabetic control group ($P = .013$) (**Table 2**).

Sperm count

The results showed a significant decrease in epididymal sperm number of diabetic control group compared to healthy control group ($P = .001$). The results also showed that the average sperm number of diabetic aer-

Table 4. The level of follicle stimulating hormone (FSH), mIU/ml; luteinizing hormone (LH), mIU/ml; testosterone, nmol/l; adiponectin, mg/l and Insulin, (mIU/L) in different groups of rats.

Groups	LH (mIU/ml)	FSH (mIU/ml)	Testosterone (nmol/L)	Adiponectin (mg/L)	Insulin (mIU/L)
HC	5.6 (±2.8)	4.4 (±1)	6.6 (±1.8)	5.6 (±2.2)	3.4 (± .52)
DC	3.9 (± .7)a	3.5 (±1.1)	4.6 (±1.6)a	1.6 (± .6)a	4.5 (± .96)a
DAT	4.7 (±1)	5.9 (±5)	5.7 (±2.3)	3.8 (±1.1)ab	3.1 (± .58)b
HAT	9.8 (±1.6)abc	4.3 (±1)	7.9 (±1.7)bc	5.3 (± .7)bc	3.3 (±.78)bc

[a]. The significant difference with healthy control group ($p < .05$). [b]. The significant difference with diabetic control group ($p < .05$). [c]. The significant difference with diabetic aerobic training group ($p < .05$).
Abbreviations: HC, healthy control group; DC, diabetic control group; DAT, diabetic aerobic training group; HAT, healthy aerobic training group.

obic training group (26 ± 13×(106)) was significantly higher than that of diabetic control group (11.75 ± 5×(10^6)) (P = .03). Furthermore, the difference between healthy control group and diabetic aerobic training group was not significant (P = .065) (**Table 3**).

Sperm viability

The mean percentage of viable sperms in the diabetic control group was significantly lower than that of the healthy control group (P = .001). Also, there was a significant increase in sperm viability in diabetic aerobic training group compared to the diabetic control group (P = .001). (**Table 3**).

Sperm morphology

There was a significant difference in the mean percentage of morphologic natural sperms between the rats of healthy control group and diabetic control group (p = 0.007), but the difference between diabetic control group and diabetic aerobic training group was not significant (P = .566) (**Table 3**).

Sperm motility

The mean percentage of sperm motility in diabetic control group was significantly lower than that in healthy control group (P = .001), while there was a significant increase in sperm motility in diabetic aerobic training group compared to diabetic control group (P = .041) (**Table 3**).

Adiponectin and hormonal levels

The mean serum testosterone (P = 0.028) and LH (P = 0.047) concentrations decreased significantly in the diabetic control group compared with the healthy control group. The differences between means serum testosterone (P = .117), LH (P = .746) and FSH (P = .596) concentrations in the healthy control group and diabetic aerobic training group were not significant.

Unlike the healthy control group, the mean serum adiponectin level of the diabetic control group decreased significantly (P = .001). Also the differences between means serum adiponectin concentrations in the healthy control group and diabetic aerobic training group were not significant (P = .269). Therefore, after a 10 week protocol, aerobic training increases serum adiponectin of diabetic rats (**Table 4**).

The mean serum insulin level of diabetic control group increased significantly compared to the healthy control group (P = .008). The difference between means serum insulin concentrations among the healthy control group and diabetic aerobic training group were also not significant (P = .12). Therefore, 10 weeks aerobic training reduced serum levels of insulin in diabetic rats (**Table 4**).

Correlation of serum adiponectin and hormonal levels

Significant correlations were observed between serum adiponectin and serum LH (r = .495, P = .049) and serum testosterone (r = .406, P = .014) in diabetic type 2 rats (**Table 5**).

DISCUSSION

The present study examined the effect of 10 weeks of aerobic training on serum levels of adiponectin and the sex hormones (testosterone, LH, and FSH) as well as sperm parameters in type 2 diabetic rats. During the last decade, diabetes and infertility have increased simultaneously[16]. However, scientific reports regarding the relationship between diabetes and infertility are limited. The results generally affirm that diabetes has a negative effect on sperm parameters[3]. According to WHO, sperm parameters include sperm count, sperm viability, sperm motility and sperm morphology[17]. Also confirmed that sperm parameters in the diabetic control group were significantly lower than those in healthy control group[18]. The proposed mechanism is such that diabetes induces testicular changes through apoptosis, atrophy of seminal tubes, decreasing the diameter of seminal tubes, and decreasing the cellular complex of spermatogenesis, and the harmful effects on the production of natural sperm and spermatogenesis[19]. Also experimental studies have shown that diabetes and insulin resistance result in a decrease in GnRH, LH, FSH, and testosterone via the effect on hypothalamic-pituitary-gonadal axis[20]. The increase of blood glucose results in a decrease in sex hormones including testosterone and sperm parameters through mechanisms such as an increase in oxidative stress in testis tissue and destroying productive cells of GnRH in hypothalamus [21]. The present study also revealed that the amount of reproductive hormones of LH, FSH, and testosterone in diabetic rats was lower than that of healthy rats. These results are in agreement with the results of Mohamed et al[22] and Erdemir et al[21]. In fact, our study supports the idea that a reduction in androgenic hormones is probably one of the main mechanisms in disrupting fertility of diabetic rats.

This experiment showed that 10 weeks of aerobic training caused a significant reduction in blood glucose of the experimental group. Physical exercise has been suggested as an effective and non-medicinal strategy in preventing and treating infertility. Observing consumed calories, adopting a healthy diet and a moderate physical activity help improve the quality of sperm[23]. Alhashem et al. also reported that fattening rats with a high- calorie diet decreased their fertility capacity and a following aerobic training improved their spermatogenesis[24]. In sum, studies have indicated that exercise at a moderate intensity can increase male fertility, probably through mechanisms such as improving endocrine system status, oxidative stress and body composition[25]. The current study indicated that aerobic training in diabetic rats increases sex hormones (testosterone and LH) significantly when compared to non-training diabetic group. In other words, a decrease in blood glucose after ten weeks of aerobic training was associated to the return of testosterone and LH hormones to normal levels and no significant difference between the healthy control group and the experimental group was observed.

The study also revealed a significant increase in sperm parameters, including sperm count and sperm viability in diabetic aerobic training group when compared to diabetic control group. The findings of the research indicate that performing exercise increases the efficiency of sperm fertility in diabetic rats probably through improving blood glucose levels and sex hormone status. In line with aforementioned findings in non-diabetic subjects[25,26] , our results showed that exercise improves fertility in diabetic rats. Taken together, our results are in agreement with the reports which state physical exercises at moderate intensity may cause improvement in metabolic function and sex hormones, including testosterone[27].

The findings also showed that adiponectin serum has positive effects on fertility. Kasimanickam and colleagues showed that the concentration of adiponectin and testosterone were greater in high fertility bulls

compared with average and low fertility bulls. Furthermore, sperm DNA fragmentation index was greater in low fertility compared with both average and high fertility bulls. They also concluded that the mRNA abundance of adiponectin and its receptors, AdipoR1 and AdipoR2, were greater in high fertility bulls compared with average and low fertility bulls[28]. Our study demonstrated that serum adiponectin levels of diabetic rats were significantly lower in comparison to healthy rats, which is confirmed by previous reports[29,30], and possibly indicating that low levels of adiponectin may be an effective factor in type 2 diabetes disease.

The effect of aerobic training on serum adiponectin levels of diabetic rats was investigated and indicated that adiponectin levels increase following a period of aerobic training. The results showed no significant difference between serum adiponectin levels of the diabetic aerobic training group and the healthy control group indicating the beneficial effects of aerobic training on adiponectin concentrations. Also, our study also showed that there is a positive and significant correlation between serum adiponectin, LH and testosterone in rats. Therefore, it seems that a decrease in adiponectin concentration due to diabetes may contribute to infertility.

The emergence of the metabolic hormone of adiponectin as a key endocrine signal is a major improvement not only in energy balance, but also in areas such as reproduction, inflammation, and immunology. Adiponectin, regulating pleiotropic, has a large number of biological functions, including gonadal steroidogenesis (8). As mentioned before, the means of testosterone, LH and adiponectin concentrations were higher after the aerobic training. These findings are in line with the findings of Kasimanickam et al (2013). They reported that adiponectin and testosterone concentrations are greater in high fertility bulls[28]. In the present study, there were improvements on fertility of diabetic rats after 10 weeks of aerobic training. Our findings indicated that in addition to changes in sex hormones and sperm parameters, the effects of serum adiponectin may also be associated with these improvements. The present study suggests that a short period of aerobic training improves the profile of sex hormones and serum adiponectin concentrations which can lead to an increase in fertility capacity of diabetic rats. Therefore, it is suggested that this research be replicated on diabetic rats with other kinds of exercises which decline diabetes effects following changes in serum levels of adiponectin and sex hormones in order to find suitable strategies in improving fertility of diabetic rats.

Also, the limitations of this study can be our diabetic model, so that streptozotocin–nicotinamide induced diabetes is a type I imitation, and this model does not exactly simulate type II diabetes in humans. Although various pathophysiological and molecular aspects of type 1 and type II diabetes are prevalent, some of the characteristics may vary and generally restrict this pattern to type II diabetes and insulin resistance.

CONCLUSIONS

Serum adiponectin, testosterone and LH concentrations were higher in trained diabetic rats. In addition, the quality of sperm regarding the parameters of count and viability were improved in the trained diabetic rats. In the present study, increasing adiponectin was associated with increased gonadotropic steroidogenesis (increased serum testosterone concentration).

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CONFLICT OF INTEREST

The authors report no conflict of interest.

REFERENCES

1. Cho N, Shaw J, Karuranga S,et al. IDF Diabetes Atlas: global estimates of diabetes prevalence for 2017 and projections for 2045. Diabetes Res Clin Pract. 2018;138:271-81.
2. Alves M, Martins A, Rato L, Moreira P, Socorro S, Oliveira P. Molecular mechanisms beyond glucose transport in diabetes-related male infertility. Biochim Biophys Acta. 2013;1832:626-35.
3. Omolaoye TS, Skosana BT, du Plessis SS. Diabetes mellitus-induction: Effect of different streptozotocin doses on male reproductive parameters. Acta histochemica. 2018;120:103-9.
4. Kuiri-Hänninen T, Sankilampi U, Dunkel L. Activation of the hypothalamic-pituitary-gonadal axis in infancy: minipuberty. Horm Res Paediatr. 2014;82:73-80.
5. Aziz NM, Kamel MY, Mohamed MS, Ahmed SM. Antioxidant, anti-inflammatory, and anti-apoptotic effects of zinc supplementation in testes of rats with experimentally induced diabetes. Applied Physiology, Nutrition, and Metabolism. 2018;43:1010-8.)
6. Dadras S, Abdollahifar MA, Nazarian H, Ghoreishi SK, Fallahnezhad S, Naserzadeh P, Jajarmi V, Chien S, Bayat M. Photobiomodulation improved stereological parameters and sperm analysis factors in streptozotocin-induced type 1 diabetes mellitus. J Photochem Photobiol B. 2018;186:81-7.
7. Straub LG, Scherer PE. Metabolic Messengers: adiponectin. Nature Metabolism. 2019 Mar;1(3):334.
8. Campos DB, Albornoz M, Papa PC, et al. Relationship between adiponectin and fertility in the female pig. Reproduction, Fertility and Development. Reprod Fertil Dev. 2015;27458-70.
9. Rodriguez-Pacheco F, Martinez-Fuentes AJ, Tovar S, et al. Regulation of pituitary cell function by adiponectin. Endocrinology. 2007;148:401-10.
10. Ocon-Grove OM, Krzysik-Walker SM, Maddineni SR, Hendricks GL, Ramachandran R. Adiponectin and its receptors are expressed in the chicken testis: influence of sexual maturation on testicular ADIPOR1 and

ADIPOR2 mRNA abundance. Reproduction. 2008;136:627-38.

11. Amaral LSdB, Souza CS, Volpini RA, et al. Previous Exercise Training Reduces Markers of Renal Oxidative Stress and Inflammation in Streptozotocin-Induced Diabetic Female Rats. J Diabetes Res. 2018;2018.
12. Kanter M, Aksu F, Takir M, Kostek O, Kanter B, Oymagil A. Effects of low intensity exercise against apoptosis and oxidative stress in Streptozotocin-induced diabetic rat heart. E Exp Clin Endocrinol Diabetes. 2017;125:583-91.
13. Taha H, Arya A, Khan AK, Shahid N, Noordin MI, Mohan S. Effect of Pseuduvaria macrophylla in attenuating hyperglycemia mediated oxidative stress and inflammatory response in STZ-nicotinamide induced diabetic rats by upregulating insulin secretion and glucose transporter-1, 2 and 4 proteins expression. Journal of Applied Biomedicine. 2018 Nov 1;16(4):263-73.
14. Afzalpour ME, Chadorneshin HT, Foadoddini M, Eivari HA. Comparing interval and continuous exercise training regimens on neurotrophic factors in rat brain. Physiol Behav. 2015;147:78-83.
15. Wang Y, Yang J, Jia Y, et al. Variability in the morphologic assessment of human sperm: use of the strict criteria recommended by the World Health Organization in 2010. Fertil Steril. 2014;101:945-9.
16. Bebb R, Millar A, Brock G, Committee DCCPGE. Sexual Dysfunction and Hypogonadism in Men With Diabetes. Can J Diabetes. 2018;42:S228-S33.
17. Hamilton J, Cissen M, Brandes M, et al. Total motile sperm count: a better indicator for the severity of male factor infertility than the WHO sperm classification system. Hum Reprod. 2015;30:1110-21.
18. Salimnejad R, Sazegar G, Mousavi SM, Borujeni MJS, Shokoohi M, Allami M, et al. Effect of Teucriumpolium on oxidative damages and sperm parameters in diabetic rat induced with streptozotocin. Int. J. Adv. Biotechnol. Res. 2017;8:1909-17.
19. Guneli E, Tugyan K, Ozturk H, et al. Effect of melatonin on testicular damage in streptozotocin-induced diabetes rats. E Eur Surg Res. 2008;40:354-60.
20. Temidayo SO, du Plessis Stefan S. Diabetes mellitus and male infertility. Asian Pac J Reprod. 2018;7:6.
21. Erdemir F, Atilgan D, Markoc F, et al. The effect of diet induced obesity on testicular tissue and serum oxidative stress parameters. Actas Urol Esp (English Edition). 2012;36:153-9.
22. Mohamed NA, Ahmed OM, Hozayen WG, Ahmed MA. Ameliorative effects of bee pollen and date palm pollen on the glycemic state and male sexual dysfunctions in streptozotocin-Induced diabetic wistar rats. Biomed Pharmacother. 2018;97:9-18.
23. Hayden RP, Flannigan R, Schlegel PN. The Role of Lifestyle in Male Infertility: Diet, Physical Activity, and Body Habitus. Curr Urol Rep. 2018;19:56.
24. Alhashem F, Alkhateeb M, Sakr H, et al. Exercise protects against obesity induced semen abnormalities via downregulating stem cell factor, upregulating Ghrelin and normalizing oxidative stress. EXCLI journal. 2014;13:551.
25. Du Plessis SS, Kashou A, Vaamonde D, Agarwal A. Is there a link between exercise and male factor infertility. Open Reprod Sci J. 2011;3:105-13.
26. Rafiee B, Morowvat MH, Rahimi-Ghalati N. Comparing the effectiveness of dietary vitamin C and exercise interventions on fertility parameters in normal obese men. Urolj. 2016;13:2635-9.
27. Seo DY, Lee SR, Kwak HB, et al. Exercise training causes a partial improvement through increasing testosterone and eNOS for erectile function in middle-aged rats. Exp Gerontol. 2018;108:131-8.
28. Kasimanickam VR, Kasimanickam RK, Kastelic JP, Stevenson JS. Associations of adiponectin and fertility estimates in Holstein bulls. Theriogenology. 2013;79:766-77. e3.
29. Ahangarpour A, Shabani R, Farbood Y. The effect of betulinic acid on leptin, adiponectin, hepatic enzyme levels and lipid profiles in streptozotocin–nicotinamide-induced diabetic mice. Res Pharm Sci. 2018;13:142.
30. Hemmati M, Asghari S, Zohoori E, Karamian M. Hypoglycemic effects of three Iranian edible plants; jujube, barberry and saffron: Correlation with serum adiponectin level. Pakistan journal of pharmaceutical sciences. 2015 Nov 1;28(6).