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Original Article

Effect of diabetes mellitus on semen quality

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Abstract: This study aimed to analyze the cause of declined semen quality in diabetic patients. A total of 157 participants were enrolled and divided into the normal group (n=70), fasting glucose impaired group (n=58) and diabetic group (n=29) based on their fasting glucose levels. Semen samples and serum samples were collected. Conventional semen parameters, including sperm concentration (SC), total sperm count (TSC) and progressive motility (PR%), were measured. Superoxide dismutase (SOD), malondialdehyde (MDA), interleukin-6 (IL-6), Tumor necrosis factor-α (TNF-α), Leptin (LEP), Zinc-alpha 2 glycoprotein (ZAG), adiponectin (ADPN) and α-glucosidase levels were determined by ELISA. Fructose and zinc levels were measured by colorimetry. Hormone levels were determined by chemiluminescence immunoassay. Compared to the normal group, the diabetic group showed significantly decreased sperm concentration, total sperm count PR%, and ZAG levels (P<0.05), increased FSH and LH levels (P<0.05), decreased T level (P<0.05), increased serum and seminal plasma MDA level (P<0.05), decreased SOD level (P<0.05), and increased seminal plasma IL-6 level (P<0.05), as well as significantly increased seminal plasma α-glucosidase, zinc and fructose levels (P<0.05). The fasting glucose impaired group showed decreased sperm PR% (P<0.05), and there was no statistical difference in the other test indexes. The decline of semen quality in diabetes patients is caused by many factors, including oxidative stress, inflammatory reactions, endocrine disorders, insulin resistance and other factors. Fertility guidance and treatment for diabetic patients requires comprehensive consideration.

Keywords: Diabetes mellitus, semen quality, inflammatory cytokines, insulin resistance, oxidative stress

Introduction

Diabetes mellitus (DM) is characterized by chronic hyperglycemia. The pathogenic mechanism is associated with glucose metabolism disorders caused by defects in insulin secretion or function. Recently, DM has emerged as the third most severe chronic disease threatening human health, following cancer and cardiovascular diseases [1]. It is estimated that more than 300 million people will be affected in 2025 [2]. In addition, the number of young people with DM shows an increasing trend. A meta-analysis suggests that the prevalence of Type-2 DM has increased by 31% among people aged 10-19 years old [3]. Notably, low fertility has been reported in 51% DM patients [4]. Therefore, reproductive problems in DM patients are of great concern, especially in people within active reproductive ages.

There is an evident relationship between reduced semen quality and DM [5, 6]. Possible mechanisms include an impaired endocrine system, insulin resistance, oxidative stress and inflammation [7, 8]. Most studies regard oxidative stress as the main reason for reproductive deficiency in DM patients [8, 9]. There is an increase in reactive oxygen species (ROS) levels and decreased anti-oxidant enzyme activity in DM patients [3, 10], which may impair spermatogenesis and induce DNA fragmentation. Chronic severe hyperglycemia and the resulting metabolic disorder may affect the function of the hypothalamic-pituitary-gonadal (HPG) axis, interfere with the endocrine system, and impair the production of steroids by Leydig cells [11]. Some studies also report that increased inflammatory cytokines and oxidative stress can impair spermatogenesis [12, 13]. However, the

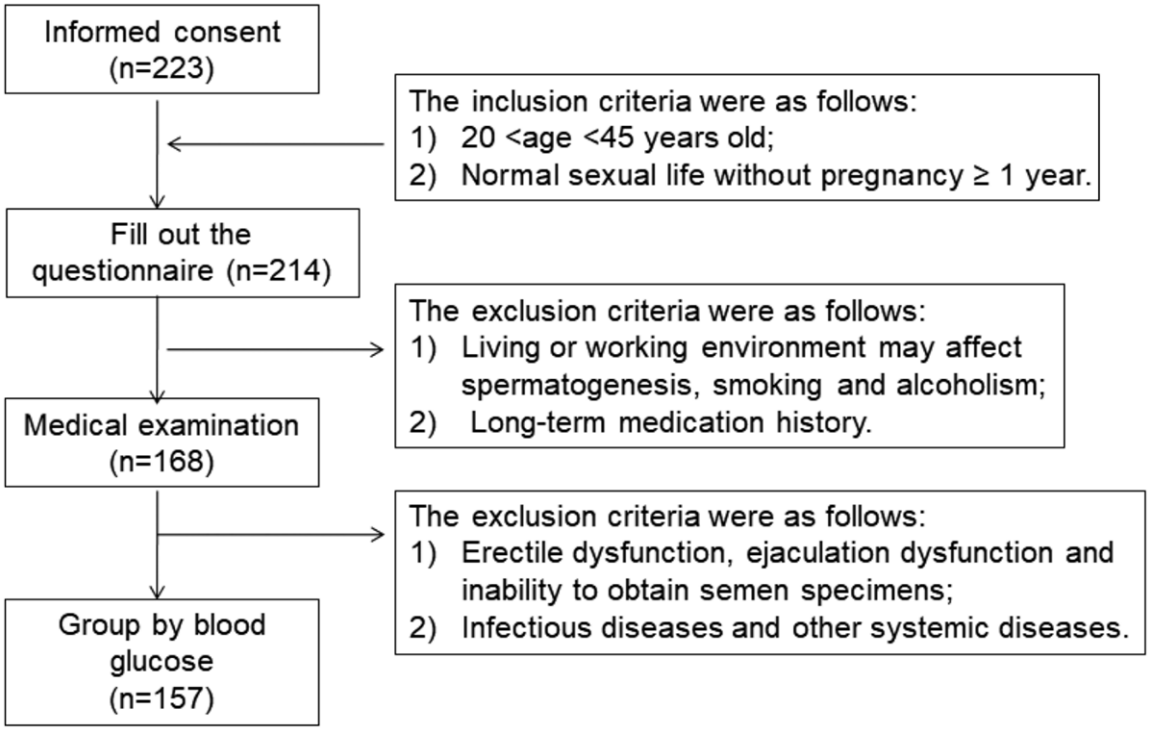

Figure 1. Study flowchart of subject enrollment.

exact mechanism of reproductive damage in DM patients remains unclear.

Although there is a lot of research on relationship of male infertility with DM, most studies have used animal models, and clinical studies on humans are rare. In addition, most experiments focus on a certain mechanism, which is one-sided. In this study, all possible relevant factors of the body that we could think of were detected to comprehensively analyze the mechanism of diabetes on male fertility. In this study, the alterations of semen quality, seminal plasma and serum pathological parameters in DM patients were analyzed. The reasons for reduced semen quality of DM patients were analyzed and discussed. Our findings may provide insight into the treatment of infertile male DM patients.

Material and methods

Patients

A total of 157 male participants (aged 20-45 years) were recruited and divided into three groups according to their fasting blood glucose levels: normal (GLU≤6.1 mmol/L, n=70), impaired fasting glucose (6.1 mmol/L<GLU≤7.0 mmol/L, n=58) and diabetic (GLU>7.0 mmol/L, n=29). The study flowchart is shown in **Figure 1**.

The inclusion criteria were as follows: 1) Aged 20 to 45 years old; 2) Normal sexual life without pregnancy ≥1 year. The exclusion criteria were as follows: 1) Patients with erectile dysfunction, ejaculation dysfunction or inability to obtain semen specimens; 2) Patients with a working or living environment that may affect spermatogenesis (such as high temperature, toxic substances) or those with smoking and alcoholism; 3) Patients with infectious diseases or other systemic diseases; 4) Patients with long-term medication history (weight loss drugs, etc.).

This study was approved by the Ethics Committee of Hebei Province Center for Reproductive Medicine. Informed consent was obtained from all participants.

Sample collection

Blood samples (3 mL) were collected from the cubital vein in the morning after overnight fasting for 8-12 hours. After centrifugation, serum samples were collected. Semen samples were

collected by masturbation into a sterile container after 2-7 days of abstinence.

Seminal analysis

Within 30 min after ejaculation, semen volume was recorded and semen analysis was conducted in accordance with the *WHO Laboratory Manual for the Examination and Processing of Human Semen 2010*. Sperm concentration, total sperm count and progressive motility (PR%) were measured on a computer assisted sperm analysis system (Jiangsu Ruiqi Life Science Instrument Co., Ltd., Jiangsu, China). Liquefied semen samples were centrifuged at 3000 r/min for 10 minutes and seminal plasma was collected for further use.

Functional assessment of accessory glands

Seminal α-glucosidase level was determined by an ELISA kit (Elisa Biotech, Shanghai, China). Seminal fructose was measured by a colorimetric method. Seminal zinc was measured by 5-BR-PAPS colorimetry.

Enzyme linked immunosorbent assay (ELISA)

The levels of superoxide dismutase (SOD) and malondialdehyde (MDA) in seminal plasma and serum were determined by ELISA kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China). Seminal IL-6 and TNF-α levels were also determined by ELISA kits (Multisciences Biotech Co., Ltd., Hangzhou, China). ZAG, LEP, ADPN levels were also determined by ELISA kits (Multisciences Biotech Co., Ltd., Hangzhou, China).

Determination of fasting blood glucose, hormones and HOMA-IR

Fasting blood glucose was measured by a glucose detection kit (Medical system Biotechnology Co., Ltd., Ningbo, China) on an ACCUTE TBA-40FR analyzer (Toshiba Medical Systems Co., Toyoko, Japan). Serum levels of follicle stimulating hormone (FSH), luteinizing hormone (LH), testosterone (T), estradiol (E2), prolactin (PRL) and insulin were determined by chemiluminescence immunoassay on a UnicelDxI 800 system (Beckman Coulter, CA, USA) with corresponding reagents (Beckman Coulter, CA, USA). Insulin resistance was calculated by the homeostasis model assessment for insulin resistance (HOMA-IR) defined as fasting blood glucose levels (FPG) in mmol/L × FINS in mIU/L/22.5. HOMAR-IR would be 1 in normal individuals and greater than 1 in patients with elevated insulin resistance.

Statistical analysis

Statistical analysis was performed with SPSS 22.0 software (SPSS Inc., IBM Corp., NY, USA). Mann-Whitney U-test was used to examine whether the results conformed to normal distribution. Non-normally distributed data were shown as median (Inter-Quartile Range, IQR) and compared by Kruskal-Wallis test among groups. Normally distributed data were shown as mean ± standard deviation (SD) and the difference between multiple groups was analyzed by one-way analysis of variance (ANOVA) followed by LSD-*t* test. Two independent sample *t*-test was used for the comparison between two groups. Statistical significance was defined when the *P* value was less than 0.05.

Results

General information of the subjects

General information of all enrolled subjects is shown in **Table 1**. The participants were aged from 20 to 45 years old, with the median age of 28.00 (6.00) years old. The abstinence time ranged from 2 to 7 days and the median value was 4.00 (2.00) days. Median FPG level was 6.13 (1.25) mmol/L among all subjects. There were no statistically significant differences in the age and abstinence time among the three groups. In contrast, statistically significant differences in FPG level, insulin level and HOMA-IR were found ($P<0.05$). These results indicated that the blood glucose levels of the three groups were statistically different, and there was insulin resistance in the DM group.

DM reduces semen quality

To determine the effect of DM on semen quality, conventional semen parameters were measured. As shown in **Table 2**, both the diabetic group and fasting glucose impaired group showed statistically significant decreases in PR% compared to the normal group ($P<0.05$). Sperm concentration and total sperm count were significantly lowered in the diabetic group ($P<0.05$), but not in the fasting glucose impair-

Table 1. Comparison of the general information of the subjects

	Normal group (n=70)	Fasting glucose impaired group (n=58)	Diabetic group (n=29)
Age (years old)	27.50 (6.00)	28.00 (6.25)	31.00 (8.500)
BMI	24.22 (4.11)	26.77 (4.86)*	27.45 (7.40)*
Systolic blood pressure (mmHg)	120.00 (10.00)	120.00 (10.00)	120.00 (19.00)
Diastolic blood pressure (mmHg)	80.00 (8.00)	80.00 (10.00)	80.00 (13.00)
Days of abstinence (d)	4.0 (2.0)	4.0 (5.0)	4.0 (2.0)
Blood glucose level (mmol/L)	5.01 (0.53)	6.30 (0.31)	8.04 (2.51)
Insulin	6.57 (4.81)	10.57 (9.94)*	14.51 (21.95)*
HOMA-IR	1.57 (1.19)	2.89 (2.83)*	5.35 (6.27)*

Note: HOMA-IR, homeostasis model assessment for insulin resistance. *P<0.05, compared with the normal group. The values are expressed as median (IQR).

Table 2. Comparison of the semen quality in the three groups

	Semen volume (mL)	Sperm concentration (10^6/mL)	Total number of sperm (10^6)	PR (%)
Normal group (n=70)	3.0 (1.5)	72.16 (52.97)	210.71 (152.95)	46.38±14.71
Fasting glucose impaired group (n=58)	3.1 (1.9)	67.1 (52.85)	210.09 (191.51)	40.17±14.78*
Diabetic group (n=29)	3.00 (2.0)	51.87 (47.91)*	144.74 (187.94)*	40.42±13.80*

Note: PR%, progressive motility. *P<0.05, compared with the normal group. The values are expressed as median (IQR) or mean ± SD.

Table 3. Comparison of the serum hormone levels in the three groups

	LH (mIU/mL)	PRL (ng/mL)	E2 (pg/mL)	FSH (mIU/mL)	T (ng/mL)
Normal group (n=70)	2.90 (1.01)	8.77 (3.55)	34.19 (13.68)	4.67 (2.87)	4.22 (1.73)
Fasting glucose impaired group (n=58)	3.72 (2.36)	9.20 (6.21)	32.80 (12.63)	5.17 (1.97)	4.18 (1.53)
Diabetic group (n=29)	4.54 (2.74)*	8.33 (4.62)	33.38 (14.19)	5.63 (4.15)*	3.40 (1.12)*

Note: FSH: Follicle-Stimulating Hormone; LH: Luteinizing Hormone; PRL: Prolactin; E2: Estradiol; T: Testosterone. *P<0.05, compared with the normal group. The values are expressed as median (IQR).

ed group (P>0.05), compared to the normal group. Semen volume had no significant changes in either the diabetic group or the fasting glucose impaired group, compared to the normal group (P>0.05). These results indicate that DM causes a decrease in semen quality.

DM causes serum hormone changes

To determine the effect of DM on the endocrine system, serum hormone levels were detected. In the diabetic group, the serum FSH and LH levels were statistically higher, and the serum T level was statistically lower, compared to the normal group (P<0.05), while E2 and PRL showed no statistically significant differences (P>0.05) (**Table 3**). However, the fasting glucose impaired group showed no statistically significant differences in serum LH, PRL, E2, FSH and T levels compared to the normal group (P>0.05). These results indicate that DM affects the endocrine system, leading to the secretion disorders of T, FSH and LH.

DM increases oxidative stress and cytokine levels

To evaluate oxidative stress and inflammation, SOD, MDA, IL-6, TNF-α were detected. The diabetic group showed significantly increased serum and seminal plasma MDA levels and significantly decreased serum and seminal plasma SOD levels, compared to the normal group (P<0.05). Whereas the fasting glucose impaired group showed a moderate increase in serum and seminal plasma MDA and decrease in serum and seminal plasma SOD without statistical significance (P>0.05) (**Table 4**).

Table 4. Comparison of the serum SOD and MDA levels and the levels of SOD, MDA and inflammatory factors in the seminal plasma of the three groups

	Serum MDA (nmol/L)	Serum SOD (U/mL)	MDA of seminal plasma (nmol/L)	SOD of seminal plasma (U/mL)	TNF-α of seminal plasma (pg/mL)	IL-6 of seminal plasma (pg/mL)
Normal group (n=70)	4.20±1.38	54.34±9.65	20.56±9.92	758.24±130.81	29.63±13.32	34.22±18.76
Fasting glucose impaired group (n=58)	4.43±2.72	52.57±8.73	34.86±17.92	711.35±139.86	31.76±15.97	36.03±18.21
Diabetic group (n=29)	5.44±2.13*	48.11±11.23*	47.11±12.48*	638.01±147.99*	33.90±16.67	45.81±19.11*

Note: MDA, malondialdehyde; SOD, superoxide dismutase; TNF-α, Tumor necrosis factor-α; IL-6, interleukin-6. *P<0.05, compared with the normal group. The values are expressed as mean ± SD.

Table 5. Comparison of the seminal plasma biochemical levels of the three groups

	α-GLU (U/mL)	Zinc (μg/mL)	Fructose (mmol/L)
Normal group (n=70)	58.71±24.77	0.63±0.13	32.0±20.54
Fasting glucose impaired group (n=58)	65.89±38.30	0.64±0.13	39.25±22.26
Diabetic group (n=29)	88.38±26.23*	0.73±0.15*	52.62±20.27*

Note: *P<0.05, compared with the normal group. The values are expressed as mean ± SD.

Table 6. Comparison of the seminal plasma LEP, ADPN and ZAG levels of the three groups

	LEP (pg/mL)	ADPN (pg/mL)	ZAG (μg/mL)
Normal group (n=70)	37.25±12.21	2175±1209	122.96±36.12
Fasting glucose impaired group (n=58)	37.50±16.13	2111±821	111.66±44.01
Diabetic group (n=29)	37.90±14.07	2015±882	99.75±42.05*

Note: LEP, Leptin; ADPN, Adiponectin; ZAG, Zinc-alpha 2 glycoprotein. *P<0.05, compared with the normal group. The values are expressed as mean ± SD.

Seminal plasma IL-6 level was significantly increased in the diabetic group (P<0.05) but not in the fasting glucose impaired group (P> 0.05) compared to the normal group. Seminal TNF-α level was slightly increased in the fasting glucose impaired group and further increased in the diabetic group. However, no statistical significance was found (P>0.05) (**Table 4**). These results indicate that DM increases oxidative stress and inflammatory reactions.

DM alters seminal plasma biochemical parameters

To evaluate the function of accessory glands, the seminal plasma α-glucosidase, zinc and fructose levels were determined. The seminal plasma α-glucosidase, zinc and fructose levels were significantly elevated in the diabetic group compared to the normal group (P<0.05) (**Table 5**). However, no statistically significant change was observed for these parameters in the fasting glucose impaired group (P>0.05). These results indicate that the functions of accessory glands may be impaired in the diabetic group.

Change of seminal plasma adipose parameters in DM patients

To determine the effect of DM on seminal plasma fat parameters, ZAG, LEP, and ADPN were measured. The seminal plasma ZAG levels were significantly decreased in the diabetic group compared to the normal group (P<0.05) (**Table 6**). However, seminal plasma LEP and ADPN showed no statistically significant changes (P>0.05). These results indicate that DM might affect semen quality by changing adipokine levels in the male reproductive system.

Discussion

DM is reported to be closely associated with male reproductive health and can induce male infertility. For example, it is reported that declined semen quality was the major cause of reduced male fertility in DM patients [14, 15]. The symptoms include decreased sperm concentration and/or sperm motility. Navarro-Casado and colleagues showed decreased T levels and reduced sperm numbers in a Streptozotocin-induced diabetic rat model [16]. Roessner *et al.* reported lowered PR% of sperm in DM patients [17]. Consistently with these findings, this study showed significantly reduced sperm concentrations, total sperm count and PR% in the diabetic group compared to the normal group, suggesting that DM impairs semen quality possibly through affecting spermatogenesis or inhibiting sperm vitality. In addition, the fasting glucose impaired group also showed significant decrease in PR%, implying that sperm vitality is more sensitive to blood glucose change.

DM may damage the HPG axis, reduce the secretion of reproductive hormones and therefore affect semen quality in patients. Sudha *et al.* showed that DM might cause damage to the HPG axis, reduce serum T, LH, FSH, PRL and testicular T levels, and consequently impair spermatogenesis in a rat model [18]. However, this study showed a significant increase

in FSH and LH levels despite a decreased T level. This may reflect a negative feedback on FSH and LH by impaired secretion of T when Sertoli cells or Leydig cells are damaged.

Insulin resistance is the major feature of type-2 DM patients, which may influence endocrine and reproductive functions through the HPG axis [19, 20]. Insulin can stimulate the secretion of T, but a negative correlation between total T and insulin resistance was found in diabetic males [19]. This study showed a significantly reduced T level in the diabetic group accompanied by an elevated insulin level and insulin resistance, suggesting a role of insulin resistance in the dysregulation of T secretion.

Most studies regard oxidative stress as the major cause of diabetic complications [8, 9]. Oxidative stress leads to a decrease in the activity and amount of antioxidant enzymes such as SOD, and increase in the amount of oxidized molecules [3, 10]. MDA is the end product of lipid peroxidation and a marker of oxidative stress. Elevated MDA levels can damage Leydig cells and affect testicular functions [21]. Shrilatha *et al*. found that the activity of antioxidant enzymes including SOD were significantly reduced in diabetic mouse model [22]. La Vignera *et al*. studied over 40 diabetic patients and found that MDA levels of patients were evidently higher than those of normal people and negatively associated with sperm concentration, total sperm count and PR% [23]. Consistently, this study showed significantly reduced SOD and elevated MDA levels in serum and seminal plasma of diabetic patients, implying that ROS may be an important factor involved in the reproductive dysfunction induced by DM. We also found that SOD and MDA levels were obviously lower in serum compare to seminal plasma, suggesting a normal function of blood-testis barrier.

TNF-α and IL-6 are two crucial inflammatory cytokines in male infertility. The former is a non-glycosylated protein secreted by multiple cell types and the latter is an immune modulator secreted by various testicular cells, including Leydig cells and Sertoli cells. Previous studies have shown a significant increase in seminal IL-6 levels in male infertile patients and a negative correlation between IL-6 levels and sperm vitality and penetration [24, 25]. Both TNF-α and IL-6 can regulate anti-oxidation and the activity of antioxidants. Importantly, an increased TNF-α level can promote lipid peroxidation to produce a large amount of ROS in sperm. Furthermore, TNF-α participates in the development of insulin resistance [26]. IL-6 is also involved in the development of insulin resistance, while IL-6 antibody treatment in diet-induced obese mice can increase insulin sensitivity [27]. Many studies have shown elevated pro-inflammatory factors in diabetic patients [12, 13]. In particular, inflammation is the major cause of declined semen quality in type-2 DM patients [3], and seminal plasma IL-6 levels in infertile males is negatively associated with sperm concentration and vitality [24]. Consistently, this study showed a statistically significant increase in the IL-6 level and a moderate increase in the TNF-α level (without significance). These results suggest that the elevation of seminal plasma inflammatory cytokine levels in diabetic patients may induce oxidative stress, augment insulin resistance, and consequently affect semen quality.

Seminal biochemical parameters reflect functions of male accessory glands. Seminal α-glucosidase is a crucial indicator of epididymis function. It catalyzes the hydrolysis of carbohydrates in glycoproteins or polysaccharides into glucose to provide energy for sperm metabolism and motility, and is reported to be negatively correlated with sperm concentration and vitality [28, 29]. In contrast, this study showed a significantly elevated α-glucosidase level in seminal plasma in the diabetic group. This contradictory finding may represent reduced utilization of α-glucosidase by poor quality semen when the function of accessory glands was normal. However, the underlying mechanism needs further investigation. Seminal plasma fructose is a key indicator of seminal vesicles, as it serves as the main energy source of sperm and positively correlated with vital sperm count, motility and fertilization competence [30, 31]. Seminal plasma fructose is synthesized from blood glucose absorbed by seminal vesicles. This study showed a significant increase of the seminal fructose level in the diabetic group, compared to the normal group, which is consistent with other studies [32]. Seminal plasma zinc represents prostate function and serves as a necessary nutrient for the maturation of sperm and a crucial factor to keep sperm vital [33, 34]. Zn-binding proteins

can reduce zinc concentration and activate acrosin. Most studies [35] suggest a positive correlation between seminal zinc concentration and sperm motility, and show a reduced zinc level in diabetic patients. Maremanda *et al*. reported abnormal testicular and epididymal cellular structures as well as reduced zinc levels in serum and seminal plasma in Streptozotocin induced diabetic rats [35]. However, in this study, the diabetic group showed a significantly increased seminal plasma zinc level. Thus the decreased sperm number may cause reduced absorption of zinc, which may account for elevated seminal plasma zinc.

ADPN, LEP and ZAG are specifically expressed in adipose tissues. ADPN is the only adipokine that is negatively correlated with the lipid content in the body. It has the function of correcting blood glucose and lipid disorders, anti-inflammation and insulin sensitization. LEP is a hormone-like protein secreted by fat cells that plays an important role in regulating food intake and energy metabolism. It can promote lipid metabolism through β-adrenergic receptors and stimulate uncoupled proteins in the inner membrane of mitochondria to increase energy expenditure. ZAG is an adipocytokine involved in the regulation of local adipose tissue [36]. It may up-regulate cAMP levels through the β-3-adrenergic receptor pathway or affect lipid metabolism by inhibiting the synthesis of key enzymes during lipogenesis. There is a close relationship between the changes of fat factors and semen quality. The lipid composition in the sperm membrane significantly changes in spermatogenesis, sperm maturation and energy, and acrosome reactions [37]. The results of this study showed that compared with the normal blood glucose group, the ZAG level of the seminal plasma in the diabetic group decreased significantly. This suggests that DM might affect semen quality through inducing the change of adipokine levels in the male reproductive system.

Conclusion

In conclusion, it was found that sperm concentration, sperm count and sperm motility were significantly decreased in diabetic patients. This may be caused by the increased levels of oxidative stress and inflammatory factors, endocrine disorders, and insulin resistance. The low fertility of male patients with diabetes is caused many factors. Thus, it should be considered comprehensively in the fertility guidance and treatment for diabetes patients.

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Disclosure of conflict of interest

None.

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