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Nuclear Factor Kappa-B Protein Levels in Sperm of Obese Men with and without Diabetes; Cellular Approach in Male Infertility

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Abstract

Objective: Although the role of obesity and diabetes mellitus (DM) in male infertility is well established, little information about the underlying cellular mechanisms in infertility is available. In this sense, nuclear factor kappa-B (NF-kB) has been recognized as an important regulator in obesity and DM; However, its function in the pathogenesis of male infertility has never been studied in obese or men who suffer from diabetes. Therefore, the main goal of current research is assessing NF-kB existence and activity in ejaculated human spermatozoa considering the obesity and diabetics condition of males.

Materials and Methods: In an experimental study, the ELISA technique was applied to analyze NF-kB levels in sperm of four experimental groups: non-obese none-diabetic men (body mass index (BMI) <25 kg/m^2; control group; n=30), obese non-diabetic men (BMI >30 kg/m^2; OB group; n=30), non-obese diabetic men (BMI <25 kg/m^2; DM group; n=30), and obese diabetic men (BMI >30 kg/m^2; OB-DM group; n=30) who were presented to Royan Institute Infertility Center. In addition, protein localization was shown by Immunocytofluorescent assay. Sperm features were also evaluated using CASA.

Results: The diabetic men were older than non-diabetic men regardless of obesity status (P=0.0002). Sperm progressive motility was affected by obesity (P=0.035) and type A sperm progressive motility was affected by DM (P=0.034). The concentration of sperm (P=0.013), motility (P=0.025) and morphology (P<0.0001) were altered by obesity × diabetes interaction effects. The NF-kB activity was negatively influenced by the main impact of diabetics (P=0.019). Obesity did not affect (P=0.248) NF-kB activity. Uniquely, NF-kB localized to the midpiece of sperm and post-acrosomal areas.

Conclusion: The current study indicated a lower concentration of NF-kB in diabetic men, no effect of obesity on NF-kB was observed yet. Additionally, we revealed the main obesity and diabetes effects, and their interaction effect adversely influenced sperm characteristics.

Keywords: Diabetes Mellitus, Nuclear factor kappa-B, Obesity Morbid, Spermatozoa, Type II

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Introduction

Obesity and diabetes mellitus (DM) are the most prevalent health threats in several countries increasing rapidly. In addition, infertility caused by male factors is associated with diabetes and overweight/obesity (1). There seems to be mediated through complex mechanisms, including chronic systemic low-grade inflammation, insulin resistance, oxidative stress, and hyperleptinemia (2). More than 30 percent of the world's population suffers from obesity. Some sexual problems such as low-quality semen, erectile dysfunction, and subclinical prostatitis have been reported in obese men (3). Similarly, DM was introduced by excess blood sugar due to impaired insulin action. Negative effects of DM on pre-testicular, testicular, and post-testicular levels have been reported (4). Patients with hypogonadism develop through central hyperleptinemia or hypothalamic pulsatile GnRH secretion changes in overweight or obese patients (5) and Leydig cell function changes (6). Mechanisms that lead to the reduction of serum levels of testosterone. In addition, glucose metabolism plays an essential role in spermatogenesis, and numerous human and animal studies have confirmed the impacts of diabetes on male sexual function, seminal fluid parameters,

nuclear DNA, and chromatin quality (7).

The adverse effects of obesity and DM on male fertility are established well (1, 2), but little information on the mechanisms of molecular approaches and infertility problems in diabetic and obese patients is available (8). Therefore, the transcription factor activity found in sperm is part of the mechanisms involved in infertility or subfertility in obese or DM patients. Spermatozoa is a unique cell that experiences two different physiological and metabolic stages during its lifetime. In the reproductive tract, spermatozoa are quiescent cells while they become metabolically active after ejaculation (9). Increased expression of pro-inflammatory cytokines in adipose tissue of obese and DM patients, blood was the first indication that inflammatory mediators and obesity/diabetes were related (7). Recently, it has been hypothesized that high levels of pro-inflammatory factors in diabetic patients lead to the activation of nuclear factor-kappa-B (NF-kB), which mediates inflammatory and metabolic responses in part through cross-talk with peroxisomal proliferator-activated receptors (PPARs) (10). NF-kB is a major transcription factor that regulates genes responsible for the innate and adaptive immune response (11). It also plays an important role in regulating apoptosis in a germ cell of human testis tissue (12, 13). NF-kB activation under DM/obesity is a consequence of mild chronic inflammation, exposure to high glucose [advanced glycation end products: (AGEs)], and free fatty acids. NF-kB signaling and production of pro-inflammatory mediators in the liver contribute to insulin resistance in the early stages of DM, Whereas NF-kB activation in adipose tissue macrophages is required to propagate inflammation and promote systemic insulin resistance in muscle and other insulin-sensitive tissues, which is required for PPARγ in adipose tissue (14). While the crucial roles of PPARγ pointed out (15), scarce report exists on the NF-kB status and activity for sperm as well as male fertility (13, 16). According to the works of literature, the activity of NF-kB in spermatozoa of men with obesity or DM which may differ from that of obese men with DM has not been studied yet. With this background, the current study aimed to monitor NF-kB presence and activity in ejaculated human spermatozoa with a focus on the situations associated with DM and/or obesity.

Materials and Methods

Study population and experimental groups

After obtaining permission from the Ethics Committee of the National Institute of Medical Research Development (NIMAD), Tehran, Iran (IR.NIMAD.REC.1398.024), samples and demographic information were collected at the Royan Institute Infertility Center, a referral infertility clinic in Tehran, Iran. This experimental study, as part of a master plan, involved 120 Iranian men who attended Royan Institute including group I: control (n=30); men with normal weight and non-diabetes mellitus; group II: obese (BMI ≥ 30 kg/m^2; n=30) and non-diabetic mellitus men (Ob); group III: non-obese diabetic men (n=30) (Nob-DM); and group IV (n=30): obese diabetic men (Ob-DM). Diagnostic criteria for non-diabetic men were glucose levels below 110 mg/dL and HbA1C levels below 5.7%. Furthermore, the use of insulin was an exclusion criterion for diabetic men. Signed informed consent was obtained from all subjects to use their semen and personal information for research purposes.

After registration, a questionnaire containing demographic information, medical and drug history, smoking status, alcohol consumption, fertility history, and surgical history was completed by each participant. Men with a history of azoospermia, genital infections, varicocele, and debilitating chronic medical diseases (cerebrovascular, cardiovascular, sexually transmitted diseases, systemic diseases, and acute infections) were excluded from the study. BMI was calculated with the formula of weight/(height^2) (kgm^{2-}) and classified into normal weight (BMI 18-25 kg/m^2) and obese (BMI≥30 kg/m^2). All participants sustain a physical examination including anthropometric measurements (height and body weight, waist circumference (WC), and hip circumference (HC). WC and HC along with the ratio of waist to HC were also measured.

Collection and analysis of semen

Semen samples were collected after 2 to 5 days of sexual abstinence according to the World Health Organization (WHO 2010). Semen volume was measured with conical graduated tubes. The CASA system [Sperm Class Analyzer Software (SCATM, Microptic, version 4.2, Barcelona, Spain)] is used to assess sperm motility and concentration. The system consisted of a phase contrast microscope (Nikon™ Eclipse E-200, Japan) with a thermal plate. The Images were captured with a video camera (Basler Vision TecnologieTM A312FC, Ahrensburg, Germany) at 50 fps and 100x magnification. Haemocytometer method with counting chamber Neubauer improved bright lines by measuring sperm concentration in million/milliliters (M/mL) (Carl Roth™, Karlsruhe, Germany) (17). A Makler chamber was used for motility scoring. In this study, we only evaluated sperm progressive and general movements (18).

Blood sampling and analysis methods

Biochemical markers such as serum glucose concentration were measured using a standard enzymatic method (Roche Diagnostics GmbH, Mannheim, Germany). Serum insulin levels were measured using an electro-chemiluminescence immunoassay (ECLIA) kit (Roche Diagnostics GmbH, Mannheim, Germany). Glycosylated hemoglobin (HbA1C) was determined using the Nyco Card Reader II analyzer according to the manufactured instructions for use. Evaluation of the homeostasis model of insulin resistance (HOMR-IR) based on the formula: fasting insulin (microU/L)×fasting glucose (nmol/L)/22.5 was calculated.

NF-kB protein analysis and Immunocytofluorescent assay

For the detection of NF-kB protein levels, the validated method by ELISA was used (19). The total protein was extracted from equal amounts of cells (~1×10^7 sperm) in all samples using phosphate-buffered (PB). Sperm proteins were collected in the supernatant and concentrations were determined by a BCA protein assay kit (Thermo Fisher Scientific, USA). After that, the NF-kB /p65 Active ELISA kit was used to measure the binding activity of free NF-kB p65 in nuclear extracts. The analysis was performed using the sandwich ELISA method, according to the manufacturer's instructions. Supernatants containing the solubilized nuclear proteins were collected, and the quantity of NF-kB in the nuclear fractions was measured with an NF-kB p65 ELISA kit, (cat. no. ab176647; Abcam, Cambridge, UK) according to the manufacturer's instructions. For protein localization by Immunocytofluorescent Assay, sperm cells were washed in phosphate-buffered saline (PBS, Sigma, USA, 1X), and a uniform smear was prepared on Poly-L-Lysine coated slides. Cells were then fixed in paraformaldehyde 4% (w/v), washed in PBS-Tween 0.05% (v/v), permeabilized with Triton X-100 (0.5%) and resuspended in 10% (v/v) secondary host serum were blocked. Finally, primary and secondary antibodies were used. Also, a slide without a primary antibody was used as a negative control for each sample (9).

Statistical analysis

Quantitative variables were presented as mean ± standard deviation (SD). Qualitative variables were presented as percentages. Data were initially tested for normal distribution using the Kolmogorov-Smirnov test, and non-normally distributed data were log-transformed before analysis. The data of four groups (control, obese, diabetic and obese and diabetic) were analyzed using the General Linear Model (GLM) method of SAS software, in which the main and reciprocal effects of obesity and diabetes on the dependent variable were investigated (SAS 9.4). Multiple comparisons were performed using the LSMEENS statement. Differences at $P\leq0.05$ were considered significant.

Results

Comparison of age, BMI, waist circumference, hip circumference, waist-to-hip ratio (WHR), semen parameters, fasting blood sugar (FBS), hemoglobin A1c (HbA1C), Insulin, and NF-kB protein activity between experimental groups were shown in Table 1. The Obese group was younger than Non-DM and Ob-DM groups (P=0.0002). BMI (P<0.001), WC (P=0.003), and HC (P<0.001) of Obese as well as Ob-DM groups were higher than control and DM groups. However, the WHR was not changed (P=0.131). Sperm progressive motility was affected by obesity (P=0.035) and type A sperm progressive motility was affected by DM (P=0.034). Sperm concentration (P=0.013), motility (P=0.025) and morphology (P<0.0001) were altered by obesity × diabetes interaction effects. All men in diabetic groups had higher FBS (P<0.0001) and HbA1C (P<0.0001) levels. The main impact of obesity on the level of Insulin was significantly visible (P<0.0001). Obesity's main effect on beat-cross frequency (BCF) was significant (P=0.018). The levels of NF-kB activity were evaluated at 0.043, 0.033, 0.029, and 0.025 ng/ml for control, obese (ob), non-obese DM, and ob-DM groups. NF-kB concentrations were negatively influenced by diabetes (P=0.019) measured by ELISA (Fig.1). Obesity did not affect NF-kB activity (P=0.248). NF-kB localization has been also shown mainly in the sperm midpiece and post-acrosomal areas (Fig.2).

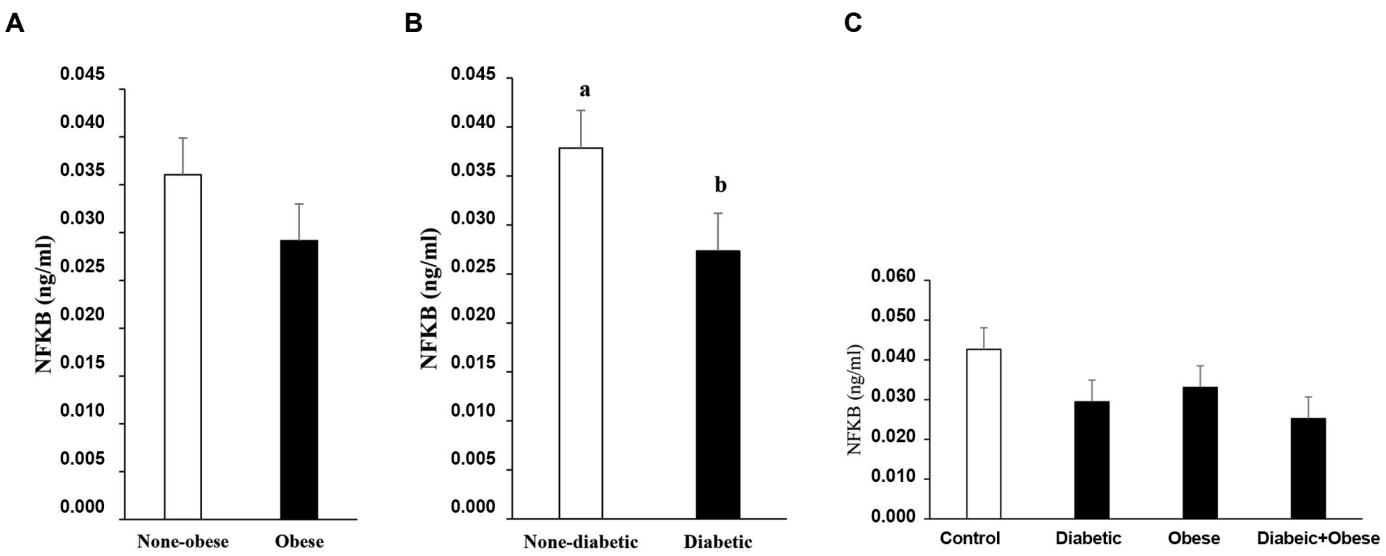

Fig.1: Nuclear factor kappa-B (NF-kB) protein activity in human spermatozoa. **A**. NF-kB protein content in obese vs. none-obese men (P=0.248). **B**. NF-kB protein content in diabetic vs. none-diabetic men (P=0.019). **C**. NF-kB protein content in experimental groups (P=0.52). [a,b]; Columns with different superscripts are different.

Table 1: Comparison of parameters in control (n=30): men with normal weight and non-diabetes mellitus; obese (BMI ≥30 kg/m^2; n=30) and non-diabetic mellitus men (Ob); non-obese diabetic men (Nob-DM; n=30); obese diabetic men (Ob-DM; n=30)

Variables	Experimental groups				P value		
	Control	Ob	Nob-DM	Ob-DM	Obesity	Diabetes	Obesity × diabetes
Age (Y)	31.30 ± 5.12[a]	32.97 ± 5.33[a]	39.33 ± 6.14[b]	39.20 ± 5.74[b]	0.455	**< 0.0001**	0.380
BMI (kg/m^2)	23.12 ± 1.26[a]	36.31 ± 4.67[b]	25.59 ± 1.89[c]	34.33 ± 4.22[b]	< 0.0001	0.687	**< 0.001**
Waist circumference (cm)	88.33 ± 4.65[a]	116.40 ± 11.91[b]	95.57 ± 8.05[c]	113.27 ± 10.39[b]	< 0001	0.223	**0.003**
Hip circumference (cm)	96.13 ± 3.70[a]	112.67 ± 22.79[b]	97.47 ± 16.99[a]	114.33 ± 7.74[b]	**< 0.0001**	0.581	0.951
Waist-to-hip ratio (WHR)	0.92 ± 0.05	1.33 ± 1.75	1.23 ± 1.54	0.99 ± 0.06	0.692	0.951	0.131
Concentration (million/ml)	75.73 ± 33.77[a]	45.04 ± 31.55[b]	51.28 ± 30.78[b]	50.77 ± 34.16[b]	0.010	0.119	**0.013**
Motility (%)	74.96 ± 14.65[a]	56.35 ± 24.57[b]	57.21 ± 25.55[b]	57.12 ± 22.61[b]	0.023	0.039	**0.025**
Morphology (%)	29.47 ± 11.33[a]	13.43 ± 13.15[b]	12.67 ± 8.77[b]	17.57 ± 12.10[b]	0.009	0.003	**< 0.0001**
Progressive motility (%)	49.64 ± 17.38	38.88 ± 25.01	38.75 ± 21.49	35.14 ± 18.89	**0.035**	0.159	0.141
Type A (%)	20.15 ± 12.15	16.29 ± 13.65	14.83 ± 10.48	12.47 ± 9.97	0.147	**0.034**	0.727
Type B (%)	28.02 ± 11.75	22.20 ± 15.87	24.39 ± 13.39	22.66 ± 11.58	0.122	0.514	0.401
Type C (%)	24.70 ± 7.93	20.71 ± 9.83	24.17 ± 20.02	23.67 ± 9.14	0.334	0.601	0.453
Type D (%)	27.14 ± 16.35	40.72 ± 24.11	52.70 ± 77.44	41.20 ± 21.44	0.894	0.098	0.111
VCL (µm/s)	55.87 ± 29.72	64.40 ± 36.38	48.73 ± 23.35	59.35 ± 27.18	0.081	0.264	0.848
VSL (µm/s)	25.78 ± 16.48	29.56 ± 17.85	21.18 ± 12.78	26.49 ± 14.69	0.115	0.183	0.790
VAP (µm/s)	35.73 ± 20.04	40.66 ± 22.56	30.36 ± 16.23	37.60 ± 19.13	0.095	0.245	0.750
LIN (%)	44.98 ± 6.52	44.63 ± 9.27	42.24 ± 10.18	43.54 ± 7.83	0.764	0.227	0.603
STR (%)	70.22 ± 7.27	70.37 ± 9.01	67.79 ± 9.38	68.75 ± 7.54	0.722	0.190	0.792
WOB (%)	63.90 ± 4.60	62.90 ± 6.27	61.47 ± 7.39	61.99 ± 6.75	0.836	0.155	0.515
ALH (µm)	2.33 ± 0.49	2.06 ± 0.73	2.06 ± 0.51	2.04 ± 0.52	0.178	0.172	0.239
BCF (Hz)	10.36 ± 4.42[ab]	12.34 ± 5.77[a]	9.01 ± 4.54[b]	11.34 ± 4.79[ab]	**0.018**	0.195	0.846
Fasting blood sugar (FBS) (mg/dl)	95.77 ± 10.65	98.70 ± 9.91	178.17 ± 76.78	156.50 ± 56.31	0.289	**< 0.0001**	0.164
HbA1C (%)	5.12 ± 0.63[a]	5.46 ± 0.59[a]	7.21 ± 1.91[b]	7.12 ± 1.69[b]	0.395	**< 0.0001**	0.315
Insulin (mU/L)	8.21 ± 3.26[a]	19.34 ± 10.68[b]	11.66 ± 12.21[a]	20.55 ± 10.46[b]	**< 0.0001**	0.443	0.803
NF-kB (ng/ml)	0.043 ± 0.032	0.033 ± 0.029	0.029 ± 0.031	0.025 ± 0.027	0.248	**0.019**	0.520

Data are presented as mean ± SD and they were analyzed using the general linear model (GLM) method. VCL; Curvilinear velocity, VSL; Straight-line velocity, VAP; Average path velocity, LIN; Linearity, STR; Straightness, WOB; Wobble, ALH; Amplitude of lateral head displacement, BCF; Beat-cross frequency, HbA1C; Glycosylated hemoglobin, NF-kB; Nuclear factor kappa-B. a, b, c; Values with different superscripts within rows differ. P values in bold were significant.

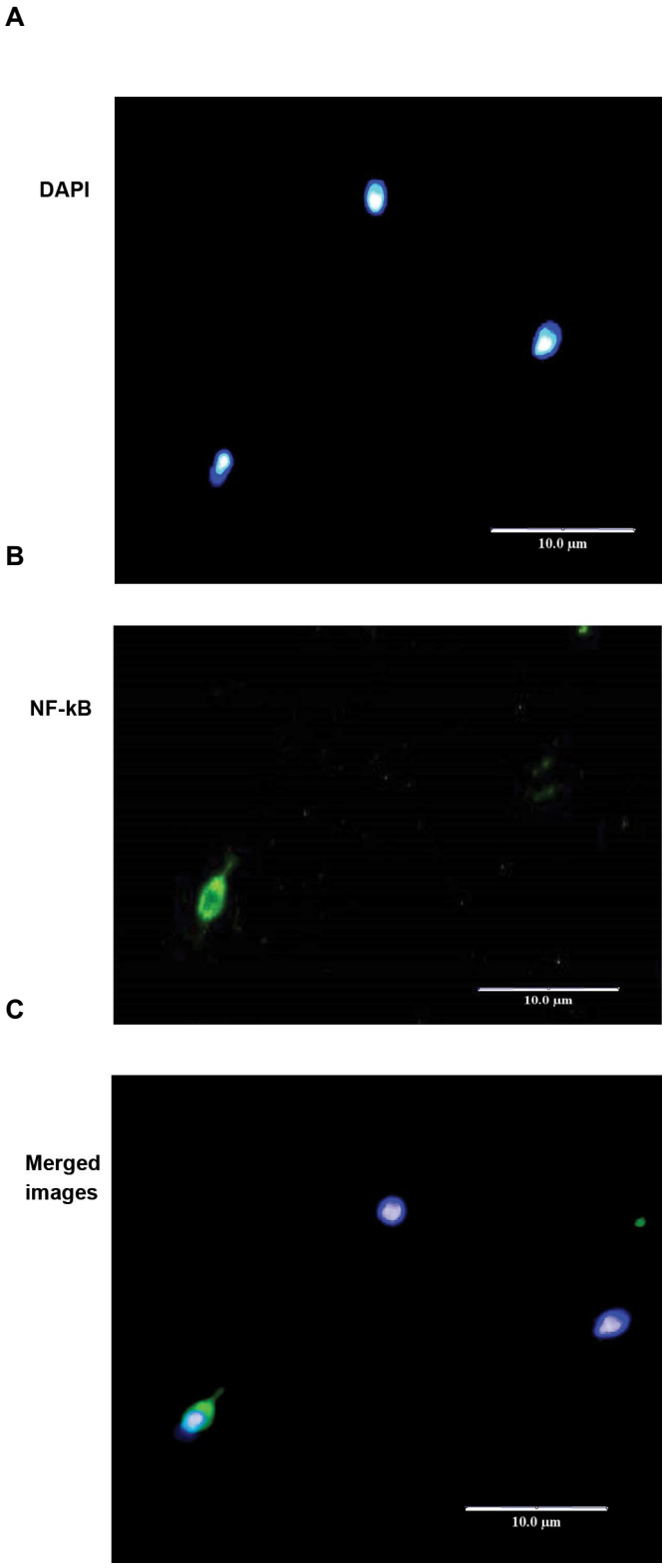

Fig.2: Nuclear factor kappa-B (NF-kB) protein localization by Immunocytofluorescent assay. **A-C.** Representative immunolocalization of NF-kB in ejaculated spermatozoa of a normozoospermic man. **A.** DAPI for nuclear staining. **B.** NF-kB detection. **C.** Merged images of A and B. NF-kB localized mainly to the sperm head and post-acrosomal regions.

Discussion

The current study provides convincing evidence for the active role of diabetes obesity or not on the level of NF-kB in the sperm of men referred to an infertility center. Based on the previous literature, obesity may not play a major role in NF-kB activity, however, in this study, the impact of diabetes on NF-kB activity is shown. While there are not many studies about the possible roles of NF-kB in male infertility, previously a published abstract represented NF-kB activity in sperm (16). For the first time in this study, by focusing on the NF-kB role in male infertility the negative impacts of obesity and diabetes on NF-kB activity in the sperm of obese and diabetic men were reported. Moreover, the results presented in the current study have shed a light on the exploration of other NF-kB roles in the sperm of diabetic men. The data of our study by providing a piece of strong evidence showed the value of monitoring men with diabetes as it may lead to their infertility. Therefore it would be valuable that refer these types of cases to infertility clinics after the diagnosis of DM. In this study, is shown that DM may exert different effects on NF-kB activity.

Recently, Zhong et al. (1) focused on the association of diabetes and obesity with sperm parameters. The mechanisms of obesity affecting semen quality include (17, 20): the first is male endocrine dysfunction, such as lower testosterone levels in obese men, which may be the main cause of decreased semen volume and total sperm concentration. The second is the damage of the inflammatory factors on the seminiferous epithelium of the testicular tissue, which ultimately leads to damage to the spermatogenesis process in obese men. Third, increasing oxidative stress may damage sperm structure and function, as high levels of reactive oxygen species (ROS) may attack sperm mitochondria and nuclei in obese men. On the other hand, DM mechanisms affecting semen quality included testicular and post-testicular levels (7). At the level of the testis, DM seems to cause: i. Increased oxidative stress by increasing the production of ROS in the seminal fluid and lipoperoxidation (LP), ii. Increasing the level of sperm DNA fragmentation, iii. Modification of sperm mitochondrial bioenergetics, and iv. End products of enzymatic glycation. In addition, possible post-testicular mechanisms may occur, as DM may cause sperm damage and prevent seminal fluid release by i. Infection/inflammation of the male parathyroid glands, where the association enhances the inflammatory response in the seminal fluid; and changes the normal parameters of sperm and causes it to increase more. Expansion of the inflammatory process and its chronicity and ii. Erectile and ejaculatory dysfunction, well-known complications of DM. It is surprising to note that NF-kB protein activity is an undertreated issue in diabetes and obese men.

While conflicting data on the effects of obesity and DM on seminal fluid volume and concentration have been reported (1), the adverse effects of obesity and DM on sperm motility were a common response. As a possible mechanism, it seems that the negative effects of the interaction of obesity and diabetes develop mitochondrial dysfunction and sperm motility reduction in the experimental groups in comparison with the normal group. Furthermore, our findings not only confirm the

deleterious effects of DM on the kinematic properties of human sperm, but also these data provide evidence that DM can effectively alter sperm motility in diabetic men with a BMI within the normal range. In an animal model, Abdel-Fadeil et al. (21) confirmed that obesity and diabetes in combination have more detrimental effects on male fertility than obesity alone in male rats. In contrast, a recent study concluded that obesity and diabetes in humans have a minor effect and are not harmful to male fertility (22). However, the important role of chronic inflammation in obesity and diabetes has been distinguished by a recent study (23).

As a possible mechanism, obesity impairs fertility and reproductive potential in men, particularly through alterations in the hypothalamic-pituitary-gonadal axis including sperm concentration, motility, viability, and normal morphology (3). Our data support this hypothesis when we compare the normal group with the obese group. Uniquely, BMI does not affect sperm quality, but rather reproductive hormone levels (24). Similarly, a meta-analysis that examined 20,367 obese and 1,386 diabetic patients reported that obesity and diabetes negatively affect sperm parameters in men and are associated with low testosterone levels (1). Although obesity, as well as diabetes, were expected to have deleterious effects, our findings in men with diabetes but normal BMI suggest that normal or low BMI may be a complication of diabetes. Considering a wide change in men's lifestyle, further investigation of molecular and cellular mechanisms in this group is necessary. For the first time in this study, while the tail is almost completely unstained in the NF-kB localization on human spermatozoa was represented in the midpiece and post-acrosomal regions. It has been hypothesized that high levels of pro-inflammatory factors in diabetic patients lead to the activation of NF-kB, which mediates inflammatory and metabolic responses in part through cross-talk with PPARs. As mentioned before, the localization of NF-kB was not reported in previous studies, however, Aquila et al. (25) and Mousavi et al. (9) studied on the localization of PPARγ in sperm and suggested a non-genomic signaling action in this particular cell type. Further studies on NF-kB seem to confirm that the multiple roles of NF-kB are location-dependent in the future.

Uniquely, Fan et al. (26) suggested that NF-kB should be considered in the chronic inflammation of obese men and they noted that signaling factors regulated by NF-kB were expressed at higher levels in the reproductive tract of obese men. In this study, NF-kB concentration in sperm was negatively affected by DM. The low level of NF-kB protein activity by ELISA in diabetic cases shows the critical role of DM on sperm. However, obesity may not play a significant role in the alternation of the level of NF-kB in sperm in our study. Similarly, a previous study using the ELISA technique to evaluate NF-kB protein activity in the sperm of infertile men investigated low levels of NF-kB in their sperm (16). On the other hand, previous studies focused on NF-kB activity in testes (13, 27) and there is little information about sperm. As a putative mechanism, increased levels of AGEs, its receptors (RAGE), oxidative stress, lipoproteins, and hyperlipidemia increase NF-kB expression through different pathways in subclinical/infertile diabetic men. Furthermore, inappropriate expression of NF-kB increases apoptosis and inflammatory process, that play a major role in cellular damage and subsequent complications (28). High levels of blood sugar cause oxidative stress and the formation of AGEs/RAGE in nerve cells, increasing glycated hemoglobin (HbAc1), and the level of stromal collagen in peripheral nerves, Schwann cells, and endoneurial vessels is another risk factor for the development of peripheral nerve damage (29). In contrast, our data support reduced NF-kB activation in sperm of DM men, which was confirmed by a previous study (16) that indicated a decrease of NF-kB activation in sperm. Meanwhile, the idea that different protein levels in different sub-fertile men serve as biomarkers is an exciting and emerging area for research and clinical studies. Hence, the cross-talk between diabetes and obesity on male infertility is important, and some biomarkers in sperm such as high mobility group protein 1 (HMGB1) (30), and mitochondrial uncoupling protein 2 (UCP2) (31) and it is considered special. PPARγ investigation may open the horizon for future interventions in diabetes and male infertility (32). Also, the level of several proteins in types of diabetes (type 1 and type 2) obesity or non-obesity is a subject that receives research and clinical attention.

Aging leads to adipose tissue dysfunction and thus systemic effects such as inflammation and low peripheral insulin sensitivity (33). Age matching of experimental groups can affect the results of such studies. Since the beginning of the survey, this has been one of our team's concerns and has been in the spotlight. There are always limitations in human research projects, and elderly men with diabetes are certainly one of them. Recently, older age in the diabetic group was shown in Iraq (22), which confirms our findings. In Iraqian study, the obese-diabetic group (43 years) was older than the control group (37 years). It is surprising to note that their control group was older than our control group. Therefore, we are faced with a challenge for age-matched of experimental groups in studies with diabetic men and controls in the infertility clinic, which needs more attention in future studies. Another limitation is the evaluation of NF-kB

protein phosphorylation in the current study, and we focused on net NF-kB protein activity by ELISA. In addition, future studies should focus on sperm DNA damage alongside NF-kB protein activity.

Conclusion

The current study indicated lower concentration and activation of NF-kB in diabetic men, while no effect of obesity on NF-kB activity was observed. Further, the present study revealed the main effects of obesity and diabetics, and their interactivity effect harmfully influenced sperm characteristics.

Acknowledgments

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Authors' Contributions

S.A.; Main project contributor, investigation, data collection and analysis, writing- original draft preparation. A.K.; Contribution in designed this study focusing on NF-kB, interpreted the experimental data and writing- original draft preparation. A.S.; Methodology, confirmed the authenticity of all the raw data, writing- original draft preparation and visualization. M.A.S.G.; Project advisor, the head of team in clinic, methodology and participants selection. V.B.; Contributed to conception of NF-kB related mechanisms and manuscript drafting and revising. A.N.N.; Project advisor and manuscript revising. V.A.; Data analysis, methodology, writing- review and editing. A.A.; Principle investigator (PI) as the young researcher and project management regarding NF-kB and PPAR-gama at Royan Institute, study design, validation, writing- original draft preparation, writing and reviewing final version. All authors read and approved the final manuscript.

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