ORIGINAL ARTICLE

The effect of streptozotocin induced diabetes on sperm function: a closer look at AGEs, RAGEs, MAPKs and activation of the apoptotic pathway

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Abstract

This study was designed to (1) investigate the possible mechanisms through which diabetes-induced advanced glycation end products (AGEs) and receptor for AGEs (RAGE) activation can affect male reproductive function; and (2) corroborate the interaction of previously established independent pathways. Male albino Wistar rats (14-weeks old) weighing 250–300 g received either a single intraperitoneal injection of streptozotocin (30 mg/kg or 60 mg/kg), represented as STZ30 or STZ60 respectively, or citrate buffer (control). Diabetes mellitus (DM) was confirmed if plasma glucose levels were ≥ 14 mmol/L after 1 week. Animals were sacrificed after 8 weeks of treatment by an overdose of sodium pentobarbital (160 mg/kg body weight). The testes and epididymides were harvested. The testes were used for biochemical and Western blot analysis, while sperm was retrieved from the epididymis and analysed with computer-aided sperm analysis. The blood glucose levels of STZ60 animals were above the cut-off point and hence these animals were regarded as diabetic. Diabetic animals presented with a non-significant increase in AGE and RAGE expression. Diabetic animals showed a significant increase in the expression of cleaved caspase 3 compared to control ($p < 0.001$), and these animals also presented with an increase in the expression of JNK ($p < 0.05$), PARP ($p = 0.059$) and p38 MAPK ($p = 0.1$). Diabetic animals also displayed decreased catalase activity accompanied by a non-significant increase in malondialdehyde levels. Additionally, there was a significant decrease in the percentage of progressively motile spermatozoa ($p < 0.05$) in diabetic animals. This study has shed some light on the interplay between DM, AGE, RAGE and mitogen-activated protein kinase signalling in the testes of diabetic rats, which can result in altered sperm function and contribute to male infertility. However, more studies are needed to better understand this complicated process.

Keywords Advanced glycation end products · Apoptosis · Diabetes mellitus · Mitogen-activated protein kinases · Receptor for advanced glycation end products and sperm

Abbreviations

AGE	Advanced glycation end product
BCA	Bicinchoninic acid
CASA	Computer aided sperm analysis
DM	Diabetes mellitus
DNA	Deoxyribonucleic acid
GnRH	Gonadotropin-releasing hormone
ICAM-1	Intercellular adhesion molecule-1
JNK	C-Jun N-terminal kinase
MAPKs	Mitogen-activated protein kinase
MCP-1	Monocyte chemoattractant protein-1
MDA	Malondialdehyde
Nf-kB	Nuclear factor kappa B
OS	Oxidative stress
PARP	Poly adenosine diphosphate-ribose polymerase
PUFA	Polyunsaturated fatty acid
RAGE	Receptor for advanced glycation end product
ROS	Reactive oxygen species
SF	Supplementary figure

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STZ Streptozotocin
TBA Thiobarbituric acid
VCAM-1 Vascular cell adhesion molecule-1

Introduction

Diabetes mellitus (DM) is an embodiment of various metabolic pathologies. It represents one of the most appreciable health hazards as its prevalence has increased from 177 to 422 million in only 12 years (2002–2014), which connotes a 60% increase [1, 2]. Infertility, a disease of the reproductive system, has become an integral part of global health as it is estimated to affect 60–80 million couples of which 50% is directly attributed to a male factor [3, 4]. Studies have shown that DM affects male reproductive function adversely. Some of the reported effects include a decrease in sperm motility [5, 6], alteration in sperm kinematic properties, increased abnormal sperm morphology [7, 8], decreased seminiferous tubule diameter, increased spermatogenic disruption [9–11] and a decreased number of Leydig and Sertoli cells [9, 12].

Baccetti et al. [13] reported that the gonadotropins released in response to GnRH were lower in diabetic men. This was accompanied by a decrease in sperm motility and a higher percentage of sperm immaturity, which indicates that DM may affect male reproduction by interfering with the hypothalamic-pituitary-testicular axis. Additionally, Jiang et al. [14] reported that streptozotocin (STZ)-induced diabetic rats displayed an increase in testicular tunnel positive cells, Bax/Bcl2 ratio and oxidative damage. In the same vein, Roessner et al. [15] reported increased cleaved caspase 3 in the ejaculate of diabetic men. These findings show that DM may affect male reproduction via apoptotic signalling. Furthermore, DM has been shown to increase testicular oxidative damage, which occurs when reactive oxygen species (ROS) exceeds the seminal antioxidant defence ability [16]. This results in cascading reactions, which subsequently lead to sperm DNA damage, mitochondrial DNA fragmentation, altered sperm parameters and ultimately male infertility [17]. Increased ROS production has also been implicated in the generation of advanced glycation end products (AGEs). AGEs are products of non-enzymatic reactions between sugars and the amino groups of proteins, lipids and DNA under hyperglycaemic conditions. AGEs can alter the normal functioning of macromolecules by generating ROS independently or by activating the receptors for advanced glycated end products (RAGE), which leads to a further increase in ROS production by these mutated proteins. RAGE, a multi-ligand member of the immunoglobulin family, interacts with specific ligands. Binding of RAGE to its ligands leads specifically to altered gene expression.

The role of AGE–RAGE activation in the induction of apoptosis has been widely evaluated. Many of these studies were performed on various cell types such as neuronal and endothelial cells [18, 19], as well as periodontal ligament fibroblasts [20]. Additionally, the binding of AGE–RAGE could trigger mitogen-activated protein kinase (MAPK) signalling and the nuclear factor kappa B (NF-κB) pathway, thereby leading to elevated production of ROS and upregulation of intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1) and monocyte chemoattractant protein-1 (MCP-1) [21]. Interestingly, AGEs have been suggested to play a role in instigating harm, as they are known to cause ROS production. Excessive ROS can subsequently lead to OS, to which sperm are highly susceptible. It is believed that AGEs may act as a mediator of damage to the reproductive system of diabetic men [22].

Although there are substantial data available on the effects of DM on sperm function, the mechanism through which the effects are exerted is less clearly understood. The present study therefore aimed to investigate the possibility of AGE–RAGE pathway activation in DM-related sperm function impairment and to furthermore corroborate the interaction of previously established independent pathways.

Materials and methods

Animals

Male albino Wistar rats (14-weeks old) weighing 250–300 g were used in this study. Animals were individually housed in standard cages in the Stellenbosch University's Animal Unit. Both room temperature (23 °C ± 2 °C) and light (12 h light and 12 h dark) were controlled. Ethical approval was obtained from the Stellenbosch University's Animal Ethics Committee (SU-ACUD16-00101). Rats were treated according to the recommendations of the Laboratory Animal Care of the National Society of Medical Research and the National Institutes of Health's Guide for the Care and Use of Laboratory Animals [23]. Animals were acclimatized for one week before treatment commenced and had free access to food [standard Epol™ rat chow; Nutritionhub (PTY) LTD, South Africa] and water.

Study design

Thirty rats were randomly divided into three groups (n = 10), namely, a control group and two treatment groups who received STZ. STZ (S0130-IG) was obtained from Sigma (South Africa), and a stock solution (30 mg/ml) was prepared by dissolving it in freshly prepared sodium citrate buffer (pH 4.5). Animals were treated with a single intraperitoneal injection of STZ, either 30 mg/kg body weight (STZ30) or 60 mg/kg body weight (STZ60), while the control group was treated with an equivalent volume (0.5 ml) of

citrate buffer. The successful induction of diabetes was confirmed after one week in animals presenting with a non-fasting blood glucose level of ≥ 14 mmol/l, using a Glucoplus™ glucometer. Glucose levels were monitored throughout the 8 weeks treatment period by measuring (non-fasting) once a week. Blood used for the weekly glucose level measurement was collected by pricking the tail vein of the restrained animal. Blood glucose levels are the mean of consecutive measurements.

Experimental procedures

After 8 weeks of treatment, animals were sacrificed by administering an overdose of sodium pentobarbital (160 mg/kg body weight). At the point of sacrifice, the control group had n = 10, STZ30 group had n = 10, while the STZ60 group had n = 8. The testes and epididymides were harvested, weighed and rinsed. The left testis was frozen at −80 °C until further analysis.

Sperm motility

The harvested left epididymis was placed in a Petri dish containing a 2 ml solution of Hams F-12 nutrient media (Sigma Chemicals, St Louis, MO, USA), at 37 °C. The mature spermatozoa were retrieved into a separate dish containing 2 ml Hams by dissecting the caudal area of the epididymis and allowing sperm to swim out for 30 s. The retrieved spermatozoa (2 μl) were infused into a chamber slide (20 μm, Leja, Netherlands) and placed on a microscope (Nikon Eclipse E200) with an in-built heating stage (37 °C). Sperm motility was measured via computer-aided sperm analysis (CASA) using the Sperm Class Analyser (SCA, Microptic, Barcelona, Spain). Various motility and kinematic parameters were analysed [24].

Biochemical analysis

Oxidative stress parameters

Catalase Frozen testicular tissue samples were pulverized in liquid nitrogen to prevent thawing and enzymatic reactions. An equal amount (0.05 g) of each sample was placed in an Eppendorf tube containing 50 mg of homogenization beads and 100 μl of cold lysis buffer (Na_3PO_4, 0.5% Triton X-100). Samples were homogenized by bullet blending at speed 9 for 3 min with a 1-min interval in-between. After bullet blending, an additional 400 μl of lysis buffer was added to allow for total tissue breakdown. Homogenates were centrifuged at 15,000 rpm for 20 min at 4 °C. The supernatants were transferred to different Eppendorf tubes and kept on ice. Tissue homogenates were diluted to 10× in deionized water and bicinchoninic acid (BCA) assay

was used to determine the protein concentration. From the diluted samples and standards, 5 μl was loaded in triplicate into UV microplate wells. Catalase assay buffer (170 μl) was added into each well using a multichannel pipette. Finally, 50 μl of H_2O_2 was added into the wells and analysis was performed immediately on a plate reader (Multiskan Spectrum) at 240 nm every 60 s over a 5-min period using SkanIt RE for MSS 2.2 (ThermoScientific™ Inc.) software.

Lipid peroxidation (thiobarbituric acid reactive substances (TBARS/MDA)

Frozen testicular tissue samples were pulverized in liquid nitrogen and 0.05 g of each sample was placed in an Eppendorf tube containing 0.05 g of homogenization beads and 0.5 ml of cold lysis buffer (0.1 M KPi, 1.15% KCl). Samples were homogenized by bullet blending at speed 9 for 3 min with a 1-min interval in-between. Supernatants were retrieved for further analysis (BCA and TBARS assays). In order to measure the MDA levels, 100 μl of standards and samples were pipetted into corresponding 10 ml glass tubes. Subsequently, 1 ml of SDS and 2 ml of 10% TCA-BHT buffer solution were added. Samples were vortexed, and after resting for 10 min, 2 ml of thiobarbituric acid (TBA) was added and vortexed again. The standards and samples were covered with marbles and incubated in a water bath (1 h at 100 °C), where after it was cooled on ice for 15 min. The standards and samples were centrifuged (3000 rpm, 15 min 4 °C) and the supernatants retrieved. From the supernatants, 250 μl of each standard and sample were loaded in triplicate into microplate wells and analysed on a plate reader (Multiskan Spectrum) within 30 min after centrifugation at a wavelength of 532 nm.

Western blot analysis

To determine the quantity of proteins in a sample through the Bradford method, 0.05–0.07 g of tissue was pulverized and placed into 700 μl lysis buffer. Seven stainless homogenization beads (1.6 mm, Next Advance Inc., USA) were added into each tube to allow for complete homogenization [25, 26]. The pulverized tissue was bullet blended at speed 8 for 3 min at 5 min interval inbetween. The homogenates were centrifuged for 20 min (15,000 rpm, 4 °C) and the supernatants were removed for further analysis. Tissue lysates were prepared by diluting the sample in Laemmli sample buffer and lysis buffer and boiling it for 5 min, where after 50ug protein/μl was separated by electrophoresis on a 12% SDS-PAGE mini-proteon gel. The running protocol consisted of an initial 10 min electrophoresis at 100 V and 200 mA, followed by 40 min at 200 V and 200 mA. Gels were activated using ChemiDoc (BioRad). Thereafter, the proteins were transferred onto a milipore Immobilon-P transfer membrane

(0.45 μm) (Immobilon®-P, Merck Millipore Ltd, Germany). Non-specific sites were blocked with 5% fat-free milk in TBS-Tween.

AGE was measured using Anti-AGE antibody (ab23722; Abcam, SA), and the expression of RAGE was quantified using anti-RAGE antibody (ab361; Abcam, SA). The apoptotic markers measured are caspase 3 (Sigma-Aldrich), caspase 7 (Abcam, SA), PARP (Cell Signalling Technology), p38MAPK (Cell Signalling Technology), C-Jun-N-terminal kinase (JNK) (Cell Signalling Technology) and IkBα (Cell Signalling Technology). A goat anti-mouse/rabbit-horseradish peroxidase-conjugated antibody (Sigma-Aldrich) was used as the secondary antibody. Proteins were detected and visualized using a ChemiDoc (BioRad, USA) and were further normalized (stain-free blots as housekeeping proteins) and analysed with ImageLab 5.0 (ImageLab software, USA). All primary antibodies were diluted in TBS-Tween in a 1:1000 ratio while the secondary antibody was diluted in TBS-Tween in a 1:4000 ratio. All data points are from independent biological repeats (n = 4–5).

Statistical analysis

GraphPad Prism™ software (GraphPad™ Software, Version 8.1.2(332), San Diego, CA, USA) was used for statistical analysis. Normal data distribution was measured using the Shapiro–Wilk, Anderson–Darling, Kolmogorov–Smirnov and D'Agostino–Pearson normality tests. When data passed all normality tests, a one-way ANOVA of variance with a Tukey's post-hoc test was performed. Where data were not evenly distributed, a Kruskal–Wallis test and a Dunns post-hoc test were carried out. A probability level of $p < 0.05$ was considered statistically significant and results are expressed as mean $\pm$ SEM.

Results

Basic biological parameters

After 8 weeks of treatment, STZ60 animals lost a significant amount of body weight compared to both control and STZ30 ($p < 0.0001$) (Table 1). Additionally, STZ60 animals presented with a significant increase in blood glucose levels compared to both control and STZ30 ($p < 0.0001$) (n = 8) (Table 1, Fig. 1).

Table 1 Basic biological parameters

Parameters	Control	STZ30	STZ60	Global p-value
Start body weight (g)	272.1 ± 7.076	277.2 ± 4.599	283.0 ± 3.692	0.3
End body weight (g)	340.7 ± 10.09	377.9 ± 8.777*	252.3 ± 11.80$^{\$\&}$	< 0.0001
Difference in body weight (g)	86.4 ± 19.32	100.8 ± 7.95	− 27.6 ± 11.39$^{*\&}$	< 0.0001
Glucose level (mml/L)	6.299 ± 0.169	6.532 ± 0.162	22.60 ± 1.009$^{\$\&}$	< 0.0001

Blood glucose levels is the mean of consecutive measurements from induction to termination

*$p < 0.05$ versus control; $^{\$}p < 0.0001$ versus control; $^{\&}p < 0.0001$ versus STZ30

Fig. 1 Non-fasting blood glucose levels of experimental animals as monitored during the 8-week treatment period
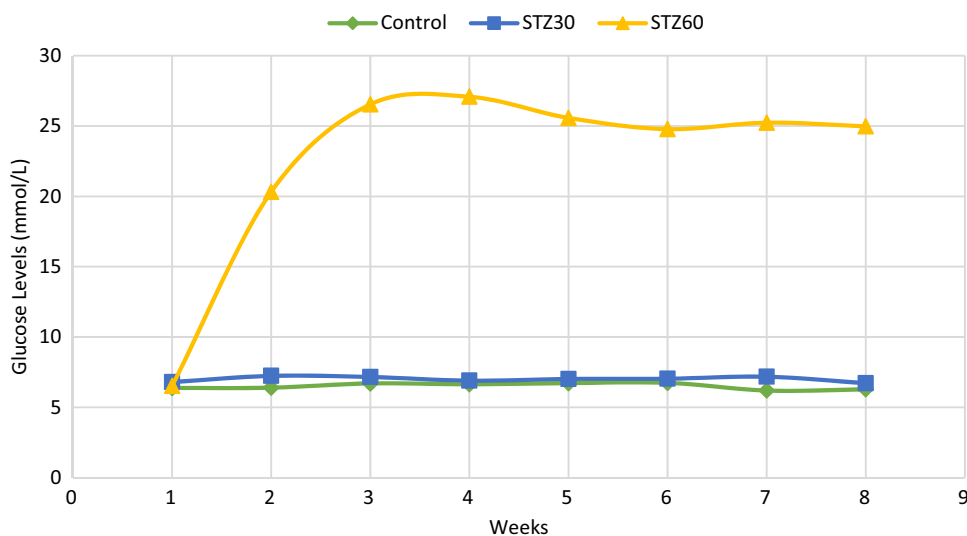
Sperm motility

STZ60 animals displayed a significant decrease in the percentages of motile and progressively motile spermatozoa compared to the control group ($p < 0.05$) ($n = 8$) [Fig. 2a, b; Supplementary Figure (SF) 1]. Although there was a decrease in the progressive motility as well as total motility of STZ30 animals compared to control, the reduction was not statistically significant. Additionally, there was a substantial but non-significant decrease in the sperm kinematic parameters (Table 2) of STZ60 animals compared to both the control and STZ30 groups respectively, [curvilinear velocity (VCL): − 30%, − 30%; straight-line velocity (VSL): − 21%, − 17%; average path velocity (VAP): − 18%, − 9%]. The kinematic parameters of STZ30 animals, despite all six being lower, remained very similar to that of control animals (Table 2).

Oxidative stress parameters

STZ60 animals presented with a non-significant decrease (− 65%) of catalase activity compared to the control group, while STZ30 animals displayed a 57.7% reduction of catalase activity compared to control ($p = 0.14$) ($n = 8$). This was accompanied by a 96.6% increase in MDA levels in the STZ60 animals and 10% increase in the STZ30 group compared to controls ($p = 0.18$) (Fig. 3a, b; SF2).

AGE accumulation and RAGE expression

Four different isoforms of AGE proteins were observed, with STZ60 animals showing a non-significant increase in accumulation of each AGE isoform—except for the 70 kDa protein—compared to control (Fig. 4a–f; SF3). Additionally, STZ60 animals displayed a non-significant increase in the total accumulation of AGE isoforms (6.4%) compared to control and STZ30 presented a 2.2% increase compared to the control group ($p = 0.6$).

Fig. 2 The effect of STZ induction on sperm motility: **a** percentage of total motility; **b** percentage of progressive motility. *$p < 0.05$ versus control. The global p for total motility $p = 0.0132$; global p for progressive motility $p = 0.0285$
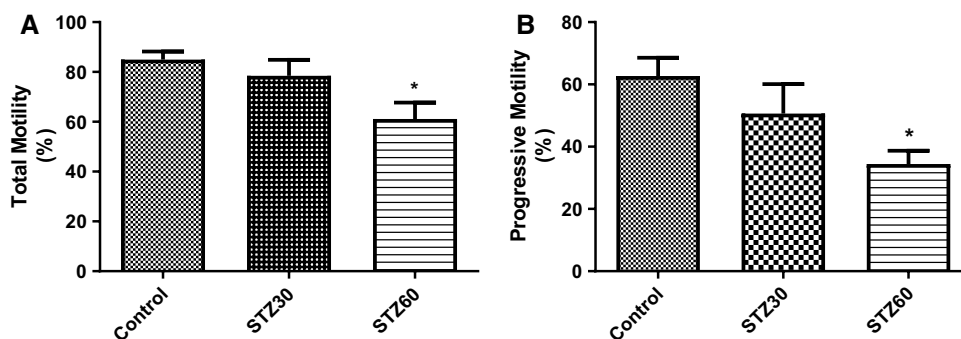
Table 2 Sperm kinematic parameters

Group	VCL (μm/s)	VAP (μm/s)	VSL (μm/s)	STR (%)	LIN (%)	WOB (%)
Control	179.0 ± 54.19	83.29 ± 26.02	50.69 ± 18.29	57.37 ± 5.192	28.65 ± 5.113	46.62 ± 6.127
STZ30	178.2 ± 86.85	79.73 ± 31.36	45.56 ± 18.83	52.46 ± 10.23	25.09 ± 7.736	44.58 ± 10.15
STZ60	124.6 ± 49.91	65.81 ± 12.74	41.53 ± 9.435	56.30 ± 7.103	28.24 ± 5.816	45.59 ± 5.362

Curvilinear speed (VCL), average path velocity (VAP), straight line velocity (VSL), straight-line index (STR), linearity index (LIN) and oscillation index (WOB)

Fig. 3 The effect of STZ induction on oxidative stress parameters. **a** The effect of STZ induction on catalase activity; **b** the effect of STZ induction on MDA levels. The global p for catalase activity $p = 0.13$, global p for MDA $p = 0.18$
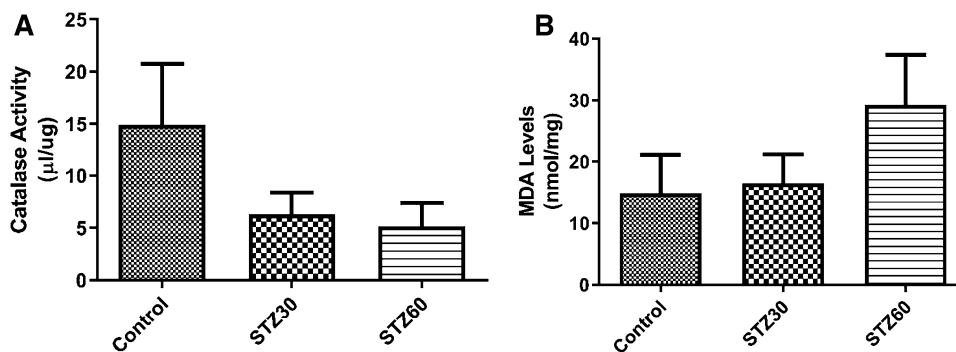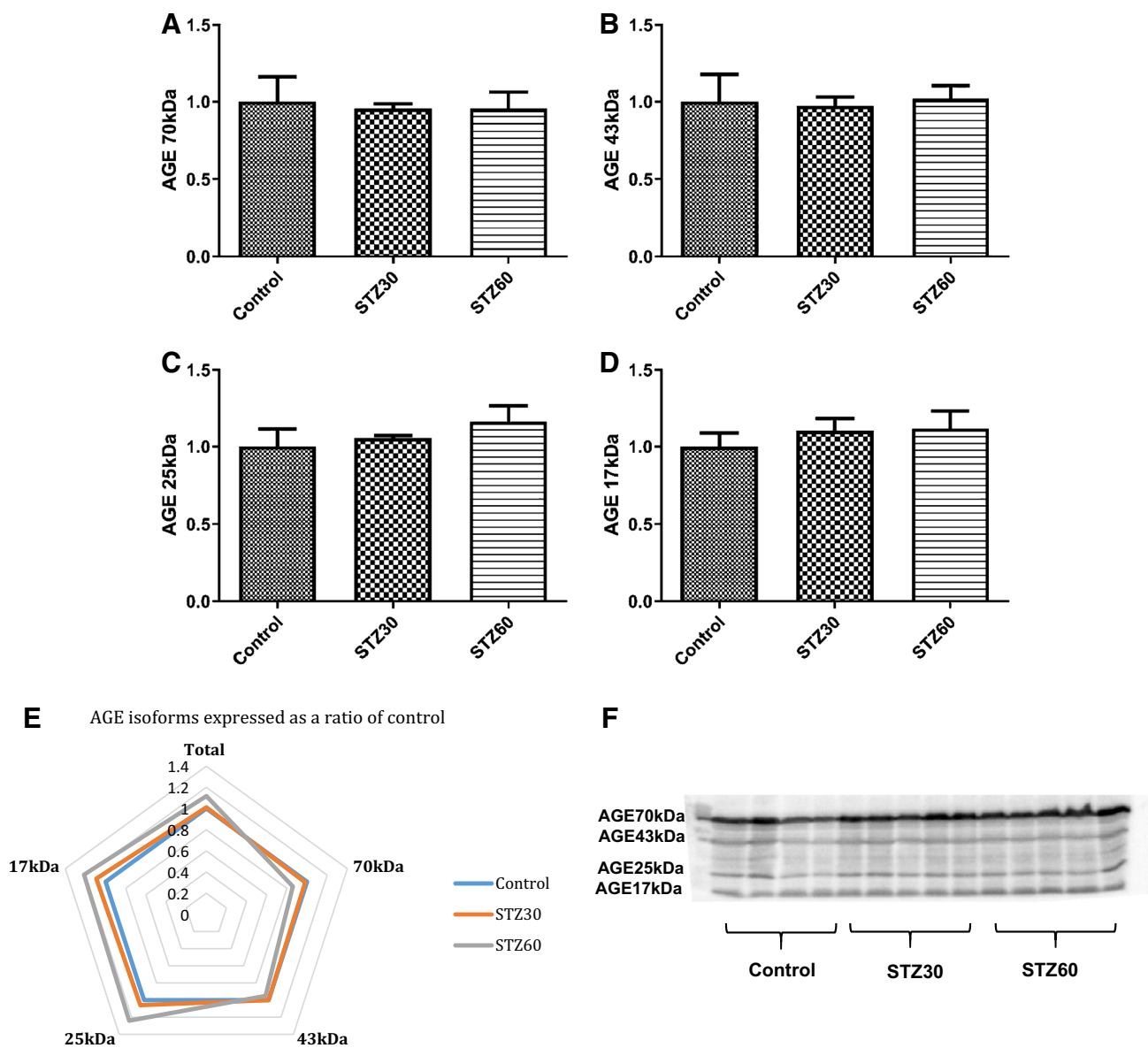
Fig. 4 The effect of STZ induction on advanced glycation end products (AGE) accumulation. Four different isoforms of AGE proteins were observed: **a** 70 kDa; **b** 43 kDa; **c** 25 kDa; **d** 17 kDa; **e** radar plot showing relative distribution of total and individual AGE isomers as a ratio of their respective controls; **f** blot (probed). The global p for **a** $p = 0.9$; **b** $p = 0.9$; **c** $p = 0.4$; **d** $p = 0.6$

In the same vein, four different isoforms of RAGE proteins were observed. The testicular tissue of STZ60 animals showed a 21.7% ($p = 0.054$) increase in the expression of RAGE (65 kDa) compared to control and a significant increase compared to STZ30 ($p < 0.05$). The total combined expression of the four RAGE isoforms was 11.7% higher than that of the control group, while STZ30 animals presented with 1.2% non-significant increase compared to the control group (Fig. 5a–f; SF4).

Apoptotic markers

STZ60 animals exhibited a highly significant increase in the expression of cleaved caspase 3 compared to both control ($p < 0.001$) and STZ30 ($p < 0.001$). Additionally, there was a 30% increase in the total expression of caspase 7 of STZ60 animals compared to control and a 20% increase in STZ30 group compared to the control group ($p = 0.056$). Although not significant, both of the STZ groups showed increased
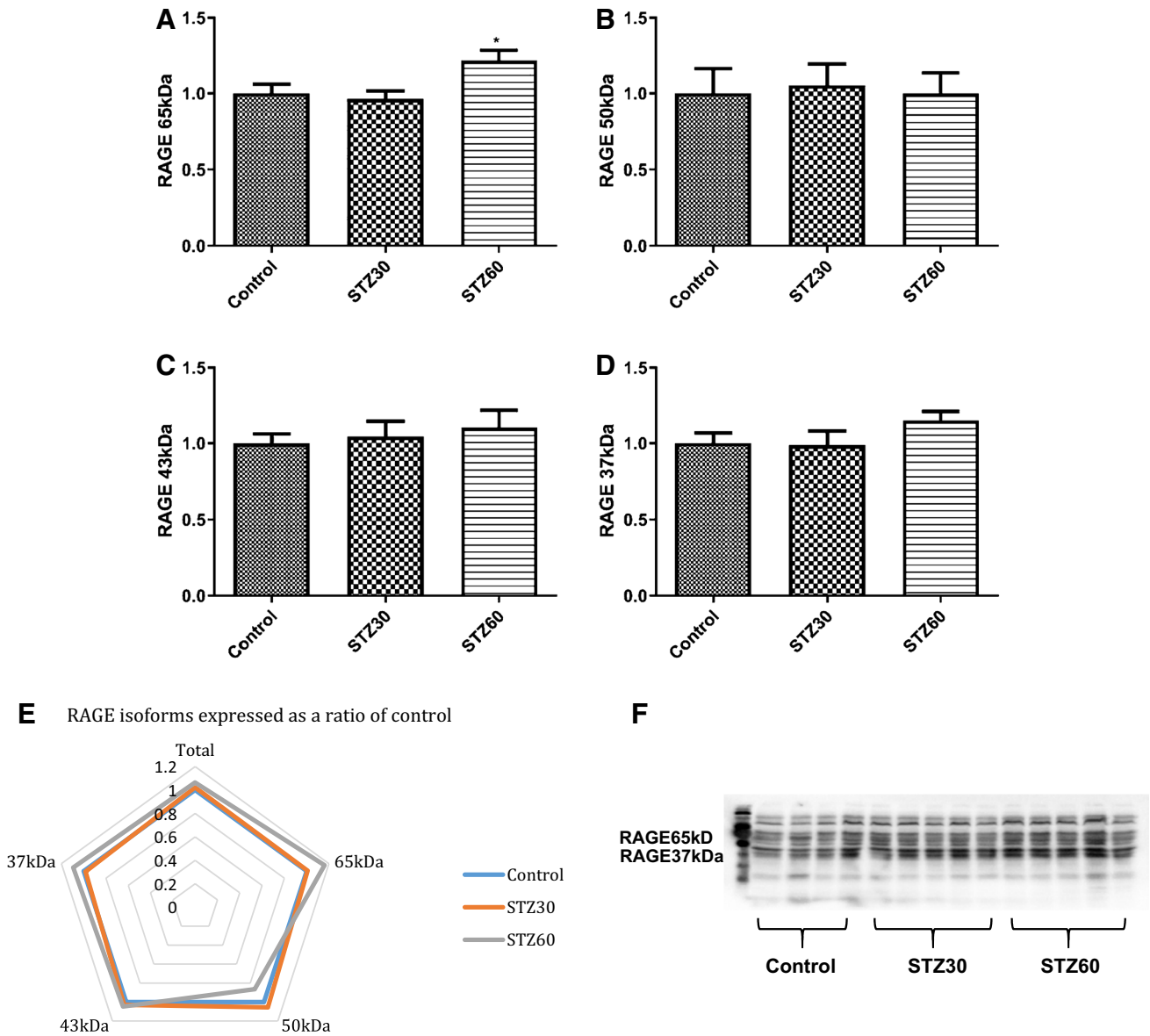
Fig. 5 The effect of STZ induction on receptor for advanced glycation end products (RAGE) accumulation. Four different isoforms of RAGE proteins were observed: **a** 65 kDa; **b** 50 kDa; **c** 43 kDa; **d** 37 kDa; **e** radar plot showing relative distribution of total and individual RAGE isomers as a ratio of their respective controls; **f** blot (probed). $*p < 0.05$ versus STZ30. The global p for **a** $p = 0.02$; **b** $p = 0.9$; **c** $p = 0.7$; **d** $p = 0.2$

PARP cleavage compared to the control ($p = 0.059$) (Fig. 6a–f; SF5).

Also observed was a non-significant gradual increase in p38MAPK expression relative to the increase in STZ concentration administered ($p = 0.2$). STZ60 animals further presented with a significant increase in the expression of JNK 46 kDa ($p < 0.05$) compared to the control group. A non-significant increase in JNK55kDa was also observed compared to control (25.6%) and STZ30 (36.3%) respectively ($p = 0.1$) (Fig. 7a–f; SF6).

Discussion

Diabetes has been shown to affect male reproductive function by altering hormone regulation and increasing OS, thereby causing testicular dysfunction and altering
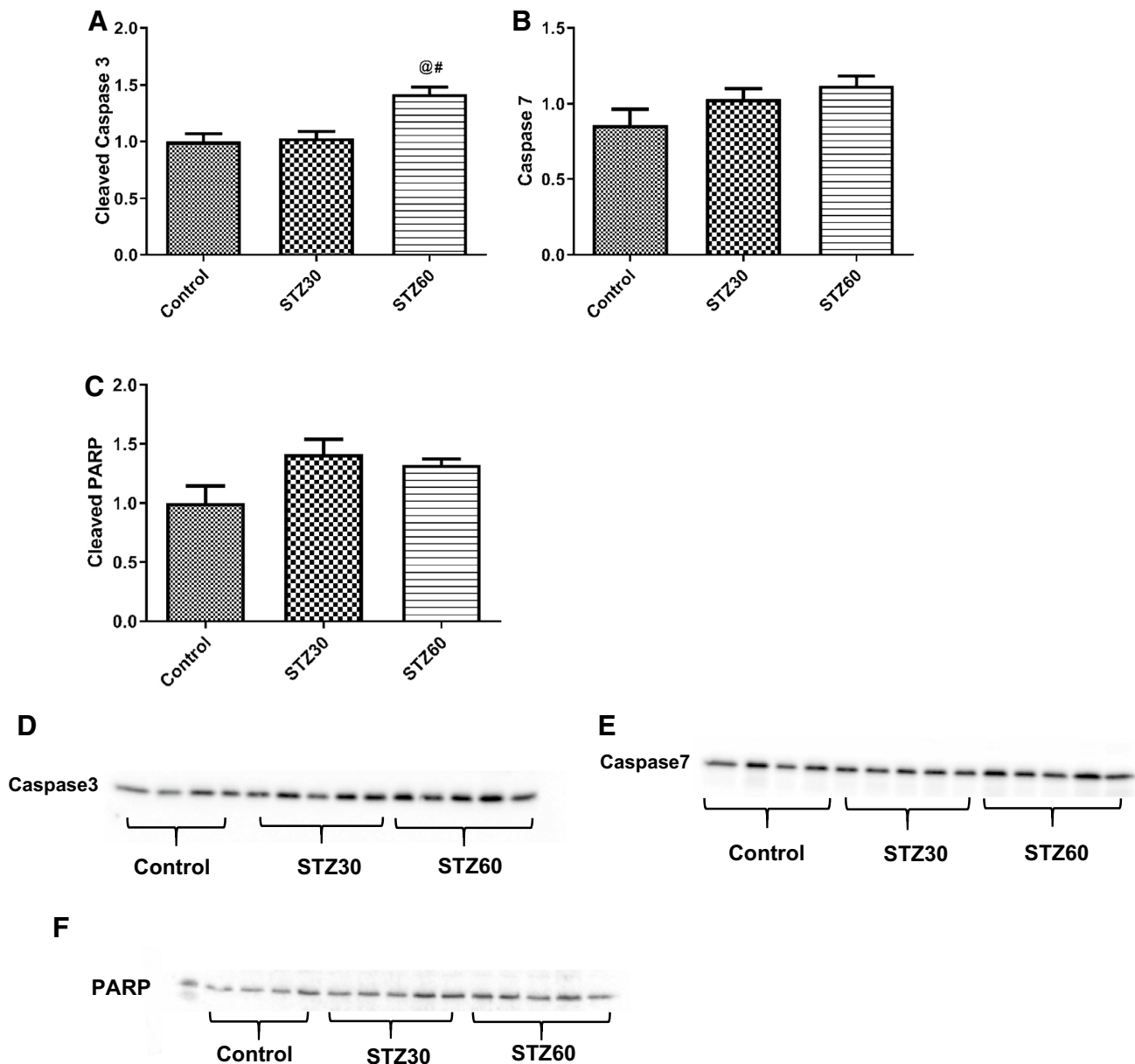
Fig. 6 The effect of STZ induction on apoptosis activation: **a** cleaved caspase 3; **b** caspase 7; **c** PARP; **d** blot for caspase 3 (probed); **e** blot for caspase 7 (probed); **f** blot for PARP (probed). $^{@}p < 0.001$ versus control; $^{#}p < 0.001$ versus STZ30. The global p for cleaved caspase 3 $p = 0.0008$, caspase 7 $p = 0.1$, PARP $p = 0.059$

spermatogenesis [10, 27, 28]. These effects subsequently result in decreased total and progressively motile spermatozoa and reduced normal sperm morphology [11, 17, 29]. However, the pathway(s) through which the effects are exerted is little understood. Hence, the current study was performed in order to investigate the possibility of alternative pathways through which DM could affect sperm function.

In the current study, STZ60 animals presented with significantly elevated blood glucose levels that were far above the normal reference range, accompanied by a substantial loss in body weight. These are typical features of type 1 DM. STZ30 animals, on the other hand, presented with only a 6.4% non-significant increase in blood glucose, which remained within the normal physiological range. Interestingly, these animals furthermore displayed a significant increase in body weight. The increase in glucose levels and decreased body weight observed in STZ60 animals might be explained by the inability to utilize the glucose derived from the food as a consequence of the ablated pancreatic β-cells and subsequent lack of insulin signalling. Hence,
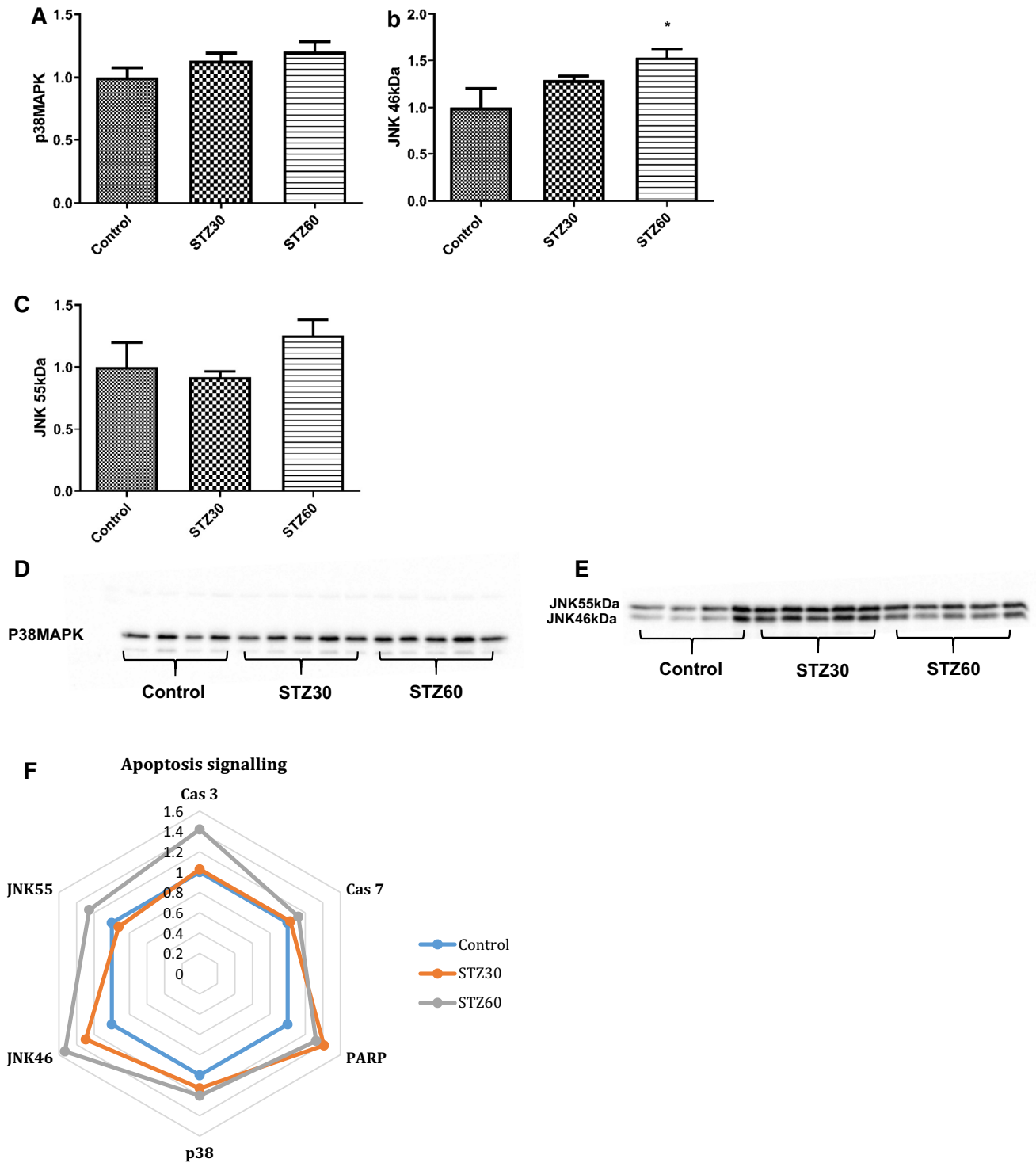
Fig. 7 The effect of STZ induction on and the MAPKs signalling: **a** p38MAPK; **b** JNK46kDa; **c** JNK55kDa; **d** blot for p38MAPK; **e** blot for JNK; **f** radar plot for all measured apoptotic and MAPK biomarkers. $*p < 0.05$ versus control; global p for p38 $p = 0.1$; JNK 55 kDa $p = 0.1$; JNK 46 kDa $p = 0.03$

the STZ60 group could be regarded as a typical type 1 DM model [30, 31].

Diabetic animals from the current study presented with a decrease in the percentage of total and progressively motile spermatozoa. This concurs with Bhattacharya et al. [32] who reported decreased sperm motility parameters in diabetic men and is also supported by Singh et al. [30] who showed impaired sperm motility in STZ-induced diabetic

rats. DM animals of the present study displayed a decrease in catalase activity and increased MDA levels. This is in agreement with the findings of Adaramoye and Lawal [33], who similarly reported increased lipid peroxidation and decreased activities of catalase, SOD and GPx in diabetic rats. Hyperglycaemia has been reported to cause increased OS in various tissues [34], including testicular tissue [35] and spermatozoa [36]. This is evidenced by another study that reported elevated MDA levels, lipid hydroperoxides and lipid peroxides in the plasma of type 1 diabetic patients [37]. In the diabetic state, lipid peroxidation can be induced by protein glycation and glucose auto-oxidation that can further lead to the formation of free radicals [38]. The damage that these free radicals impose on cells may be quantitatively determined by measuring MDA levels, which is a product of lipid peroxidation [39]. The increase in lipid peroxidation may be the result of the imbalance between the oxidant and antioxidant activities. This is supported by Buttke and Sandstrom [40] who reported that under an elevated OS status, ROS cause cellular injury via several mechanisms, including lipid peroxidation and oxidative damage of proteins and DNA. These findings are backed up by other studies which also reported a relationship between ROS and DNA damage [41, 42].

Furthermore, an increase in the production of ROS has been shown to cause the non-enzymatic and progressive glycation of proteins with the consequently increased formation

Fig. 8 The proposed mechanisms through which diabetes may affect male reproductive function. ↓ = decrease, ↑ = increase. In DM, there is altered antioxidant enzyme activity (↓Catalase) which may result in OS. The development of OS can on one arm instigate the breakdown of polyunsaturated fatty acids (MDA) and indirectly increase AGE formation. The peroxidation of PUFA attracts more ROS formation, which can activate apoptotic induction, resulting in impaired sperm function. Due to the presence of RAGE ligand, AGE, binding occurs. The AGE–RAGE complex initiates the activation of the MAPKs, which may induce apoptosis by increasing the cleavage of PARP, hence resulting in nDNA damage. All these effects collectively result in reduced male fertility

of glucose-derived AGEs. The modified proteins further cause an increase in ROS formation and aggravation of OS [43, 44] after binding to the ligand-receptor, RAGE.

AGE–RAGE activation has been implicated in the development and progression of several diseases, both metabolic and non-metabolic. This is evidenced by Chen et al. [18] who showed that the excessive production of oxidants in the endothelial progenitor cells (EPCs) resulted in RAGE-mediated AGE accumulation with resultant inhibition of cell proliferation, migration and adhesion, which were all attenuated after RAGE blockage. In the same vein, inhibition of RAGE aided the protective effect of glucagon-like peptide-1 on AGE-induced apoptotic neuronal cells [45]. Observed in the current study is an increase in the expression of RAGE in the testes of diabetic animals. This result concurs with Mallidis et al. [46] who reported increased RAGE, not only in the testes but also in the epididymis, sperm and seminal plasma of diabetic men. This study further reported that the increased RAGE expression contributed to the elevated levels of damaged nDNA observed in the spermatozoa.

An association has been reported between AGE–RAGE activation and MAPKs signalling [47–49]. MAPKs are activated in response to external stimuli. Upon external stimulation, mitogen-activated protein kinase kinase kinase (MAPKKK) is phosphorylated, thereby activating two different subgroups of mitogen-activated protein kinase kinase (MAPKK) (MKK3/MKK6 and MKK4). These dual kinases phosphorylate and activate p38 MAPK and JNK [50]. P38MAPK and JNK have been implicated in the development and progression of human diseases. Adhikary et al. [51] described the accumulation of p38 MAPK in the interstitial macrophages and myofibroblasts in the kidneys of both diabetic male mice and human. It is associated with increased HbA1c levels and albuminuria and ultimately lead to interstitial fibrosis. Additionally, p38 MAPK and JNK accumulation have been associated with apoptosis [52, 53]. Another study reported the activation of p38MAPK, followed by stimulation of cytochrome c release, caspase 8 and 3 activation, PARP cleavage and DNA fragmentation in induced apoptosis of human promyelocytic leukaemia HL-60 cells. The apoptotic events were repressed when p38 was blocked, which suggests that p38 phosphorylation occurred prior to caspase activation [54]. It was also showed that p38 MAPK is an instigator of Bcl2-x (Bax) activation and mitochondrial apoptosis in primary keratinocytes [55]. In the current study, we investigated if diabetes can affect male reproductive function through the p38 MAPK-apoptotic pathway. Similar to the previous studies, there was an increase in the level of JNK and caspase 3 and an increasing trend in p38 MAPK, caspase 7 and PARP cleavage in the testes of diabetic rats. The activation of p38 has been described to phosphorylate, activate and translocate Bcl2, Bax and Bcl-xl. The translocation causes a change
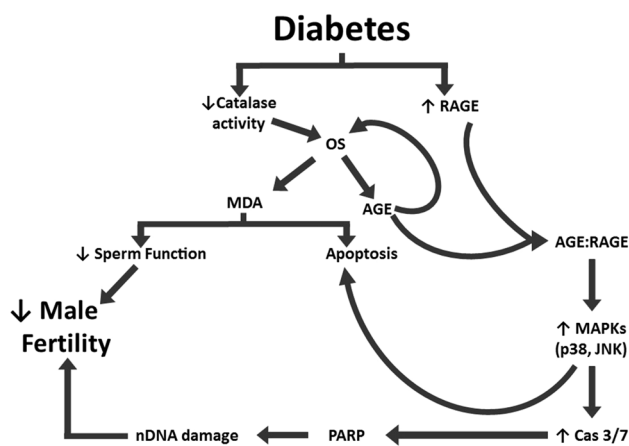
in the mitochondrial membrane potential and thus lead to cytochrome c release, which further activates the cleavage of pro-apoptotic caspases. Additionally, JNK has been shown to induce apoptosis through FASL signalling. Increase in JNK expression stimulates FAS/FASL and subsequent phosphorylation of Bid (a Bcl-2 family) and the release of cytochrome c, or it recruits caspase 8 through adaptor protein FAAD and thus cleaves caspase 3. Jiang et al. [14] reported activation of the apoptotic pathway, as evidenced by increased expression of cleaved caspase 3 and caspase 8 and subsequently altered spermatogenesis. Additionally, Metzler et al. [56] reported that spermatocyte loss in AZFc-deleted patients occurs through apoptotic pathway activation. Furthermore, a significant association was found between caspase 3 activation and increased sperm DNA fragmentation [57]. In corroboration with previous studies, the current study suggests a possible pathway and association between DM and male infertility, as explained in Fig. 8.

The current study highlighted the possible inter-connected mechanism through which diabetes may confer its impact on the male reproductive function, which may further contribute to male infertility (Fig. 8). We therefore conclude that there is an association between AGE–RAGE activation and MAPKs signalling. The increase in the expression of JNK and caspase 3 and the non-significant increase in the expression of p38 MAPK, caspase 7 and PARP cleavage in the testes of diabetic rats reveal a deadly liaison, which can result in altered sperm function and may further contribute to male infertility.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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