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High levels of bisphenol A among infertile men can impair spermatogenesis by oxidative stress and elevated levels of microRNA-337

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Abstract

Background Studies have shown that Bisphenol A may interfere with the process of spermatogenesis and result in a decrease in the quality of semen. Nevertheless, the fundamental processes remain unclear. This study was done to investigate the connections between exposure to Bisphenol A, spermatogenesis with microRNA-337, and malondialdehyde in infertile men.

Methods This study was a case–control study on 73 participants. Infertile group (1a): azoospermia ($n=16$), infertile group (1b): oligozoospermia ($n=22$), and control group (2): normospermic ($n=35$) were enrolled in this study. Full history, local examination, semen analysis, and urine and blood samples were taken from all participants. Urinary Bisphenol A, malondialdehyde, and serum microRNA-337 were measured.

Results The mean Bisphenol A level in azoospermia group shows statistically significant increase comparing to fertile control group. The mean microRNA-337 level in oligozoospermia and azoospermia group shows statistically significant increase comparing to fertile controls. The mean malondialdehyde level in infertility groups shows statistically significant increase comparing to fertile control group. No linear correlations were recorded between Bisphenol A levels with semen quality parameters, hormonal profile, and microRNA-337.

Conclusion While there is no significant change in the levels of Bisphenol A between normal fertile males and infertile males with oligozospermia, a significant elevation in the BPA level was observed in infertile males with azoospermia. A significant upregulation of the miRNA-337 gene expression in infertile males either oligozospermia or azoospermia was also observed. In addition, lipid peroxidation as evident by the significant elevation of MDA levels was marked among infertile patients.

Keywords Bisphenol A, Oligozoospermia, Azoospermia, MicroRNA-337, Oxidative stress

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1 Background

Infertility could be considered when a couple has been trying to conceive for at least a year with frequent, unprotected sexual intercourse without success. According to Boivin et al., male factor may account for up to half of the infertility cases, which occur in an estimated 15% of couples. Oftentimes, all causes of male infertility could not be identified [1].

Some research has linked environmental toxins, such as those that alter the endocrine system, to male infertility [2]. Epoxy resins and polycarbonate plastics are two of the most common places to find the ubiquitous BPA, a chemical molecule whose principal purpose is to disrupt endocrine systems [3]. BPA has recently come to light as a major concern for human health as a result of exposure via tainted food and water, skin contact, and air pollution [4].

Several studies have found that infertile males exposed to BPA showed a negative link between the amount of BPA in their urine and the quality of their semen including sperm concentration, total sperm count, progressive motility, and normal forms of sperm [5, 6].

Since several microRNAs are involved in every step of spermatogenesis, it stands to reason that male infertility issues would stem from any interference with their control. A number of diseases, including cancer and neurological problems, have been linked to changes in microRNA levels, including microRNA-337, according to earlier studies. Khazaie and Nasr Esfahani had looked at the function of these microRNAs in relation to infertility [7].

The study's overarching goal was to determine if there was a connection between the relation between infertility of adult Egyptian males and their urinary BPA, MDA, and miRNA-337 levels.

2 Methods

2.1 Study population and sample

This study was a case–control study conducted from February 2022 to June 2022 in the Andrology, Sexology, and STIs outpatient clinic at Beni-Suef University Hospital. The study included males with idiopathic infertility aged 18–50 years. We excluded people living in cities who have regular or heavy exposure to BPA at work or who have any other condition affecting the epididymis or vas deferens.

The study participants were allocated to either group (1): A sample of 38 eligible infertile subjects. Then subdivided into: (1A): non-obstructive azoospermia ($n=16$), (1B): oligozoospermia ($n=22$), and group (2): control normospermic males ($n=38$). The controls consisted of physically fit males with typical sperm characteristics, who had successfully conceived at least one healthy child in the previous year without the use of assisted reproductive methods.

Patients received a standardized analysis of their ejaculate, following the standards established by the World Health Organization [8].

To rule out non-idiopathic infertility, all the participants were subjected to a thorough medical history, a detailed family history, a personal history of infertility, a sexual history, a residence status (urban/rural), a chronological record of all surgeries, and full genital and general examination.

2.2 Semen analysis

After the recommended 2–5 days of abstinence from sexual activity, the semen samples were taken. Analyses of the semen were carried out after the samples had been liquefied at 37 °C for 30 min. The micro-cell slide and computer-aided semen analysis (CASA, version 6.3-SCA®, Microptic, S.L., Barcelona, Spain) were used for this purpose, following the procedures outlined in the 6th edition of the laboratory manual for the examination and processing of human semen, which is sponsored by the WHO [8].

2.3 Hormonal evaluation

The ELISA kits for serum FSH, LH, total testosterone, prolactin, and estradiol (E2) were obtained from different sources. The ELISA biochemical kits for serum testosterone were purchased from IBL, located at Flughafenstrasse, 52a, Hamburg D-22,335, Germany. The estradiol kits were obtained from Diametra, located at 20,090 Segrate Milano, Italy. The kits for prolactin, LH, and FSH were obtained from Pishtaz Teb, a company based in Zaman Diagnostics, USA.

MDA measurement from urine samples: MDA was extracted from the samples, using BioVision's MDA A ELISA Kit (Catalog # E5822-111) S. Milpitas Blvd., Milpitas, CA 95035 USA, www.biovision.com/tech@biovision.com.

BPA measurement from urine samples: Bisphenol A was measured in urine samples that were thawed at room temperature. BPA was extracted from the samples three times, using BioVision's Bisphenol A ELISA Kit (Catalog # E4722-100) S. Milpitas Blvd., Milpitas, CA 95035 USA, www.biovision.com/tech@biovision.com, quantitative determination of BPA, Detection Range: 0.4–30 ppb, Sensitivity: 0.2 ppb.

Measurement of miRNA-337 level in blood: Blood samples used to assay the miRNA-337 level in the sample using All-in-One™ miRNA qRT-PCR Detection Kit 2.0 for quantitative detection of mature miRNA, Cat. No. QP116, used in combination with the All-in-OneTM miRNA qPCR primers purchased from Gene Copoeia,

Inc. 9620 Medical Center Drive, #101 Rockville, MD 20850; USA.

2.4 Primers

U6 (Internal control).

Forward, 5′-CTCGCTTCGGCAGCACA-3′

Reverse, 5′-AACGCTTCACGAATTTGCGT-3′

miR-337.

Forward, 5′-ACA CTC CAG CTG GGC TCC TAT ATG ATG C-3′

Reverse, 5′-ACT CCA CGA CAC CAG TTG AG-3′

2.5 Statistical analysis

The collected data were coded then entered and analyzed using the SPSS version 21 for Windows 10. The following tests were used: Kolmogorov–Smirnov test and Shapiro–Wilk test, cross-tabulation and Chi-square test ($\chi 2$), Student's t-test, Mann–Whitney test, F-test (ANOVA), Kruskal–Wallis H-test, and Pearson's correlation analysis. P-values equal to or less than 0.05 were considered statistically significant.

2.6 Ethical considerations

The study received approval from the ethical committee of the Faculty of Medicine, our university, with an assigned ethical approval number (031-020-23). Prior to recruiting participants for the study, we ensured that all individuals provided informed written consent. We thoroughly explained the objectives of the work to them. The confidentiality of the data base was ensured (Figs. 1 and 2).

3 Results

3.1 Baseline data of the included participants

The mean age of patients in oligozoospermia group was 33.68 ± 6.26 years; while in azoospermia group, the mean age was 39.06 ± 9.67 years with a significant difference between all groups (*P*-value < 0.001). The majority of patients with azoospermia and oligoozospermia were living in rural areas while the control group were in urban areas; however, no significant differences were observed between both groups (*P*-value 136). The average BMI was within normal in all groups with no significant differences (*P*-value 0.039) (Table 1).

3.2 History and examination of the studied cases

All patients with oligozospermia had no past medical history, and 2 (12.5%) patients with azoospermia were hypertensive. No significant differences were observed between the three groups regarding past medical history (*P*-value 0.139). Smoking was reported by 7 (43.8%) patients in azoospermia group and 10 (45.5%) patients in oligoozospermia group with significant differences between the three groups (*P*-value 0.018). History of drug abuse was reported by two patients in oligozospermia and azoospermia groups (*P*-value 0.845). (Table 1).

Examination revealed that all patients in control group had normal size testis while the majority in azoospermia and oligozospermia groups had moderate sized testis (62.5 and 52.4%, respectively) (*P*-value < 0.001). Vas and epididymis were normally felt in all patients in the control

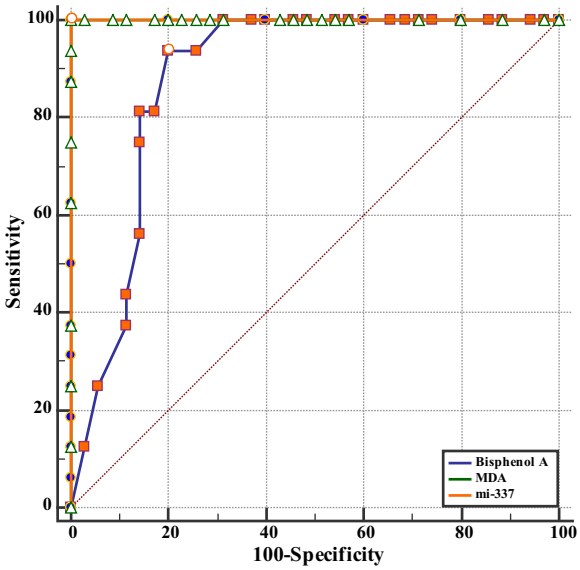

Fig. 1 Receiver operating characteristic curve for prediction of azoospermia (compared with control) from mi337, Bisphenol A, and MDA

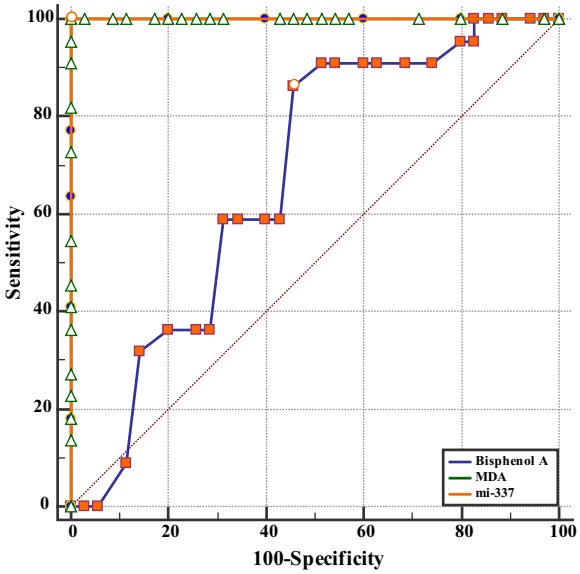

Fig. 2 Receiver operating characteristic curve for prediction of oligo (compared with control) from mi337, Bisphenol A, and MDA

Table 1 Baseline characteristics and clinical data of the studied groups

Items	Control (no = 35)	Azoospermia (no = 16)	Oligozospermia (no = 22)	*P*-value
Age (mean ± SD)	42.2 ± 7.5a	39.1 ± 9.6ab	33.7 ± 6.2b	0.001*
Residence	20(57.1%)	6(37.5%)	7(31.8%)	0.136
Urban	15(42.9%)	10(62.5%)	15(68.2)%	
Rural				
BMI	23.2 ± 2.2	24.5 ± 2.5	22.6 ± 1.9	0.039
Chronic diseases				
No	28(80.0%)	14(87.5%)	22(100.0%)	0.192
DM	2(5.7%)	0(0.0%)	0(0.0%)	
HTN	2(5.7%)	2(12.5%)	0(0.0%)	
DM + HTN	3(8.6%)	0(0.0%)	0(0.0%)	
Smoking	27(77.1%)	7(43.8%)	10(45.5%)	0.018*
Drug abuse	5(14.3%)	2(12.5%)	2(9.1%)	0.845
Testes				< 0.001*
Normal	35(100.0%)	2(12.5%)	8(38.1%)	
Small	0(0.0%)	4(25.0%)	2(9.5%)	
Moderate	0(0.0%)	10(62.5%)	11(52.4%)	
VAS				0.050
Normally felt	35(100.0%)	14(87.5%)	22(100.0%)	
Felt unilaterally	0(0.0%)	2(12.5%)	0(0.0%)	
Epididmys				0.053
Felt	35(100.0%)	13(81.3%)	22(100.0%)	
Soft	0(0.0%)	1(6.3%)	0(0.0%)	
Hard	0(0.0%)	2(12.5%)	0(0.0%)	
Cord				< 0.001*
Normal	35(100.0%)	7(43.8%)	15(68.2%)	
Thick	0(0.0%)	9(56.3%)	7(31.8%)	

P*-value ≤ **0.05 is considered significant by (Chi-square test)

and oligzospermia groups, while in azoospermia group, the vas was felt unilaterally in (12.5%) of patients (*P*-value 0.050), and the epididymis was hard in (12.5%) and soft in (6.3%) in azoospermia group (*P*-value 0.053). The cord was thick in (56.3%) in azoospermia group and (31.8%) in oligozospermia group (*P*-value < 0.001) (Table 1).

3.3 Laboratory investigations of the included participants

Hormonal profile was within normal for all patients in the three groups. There were no significant differences between all groups regarding serum FSH (*P*-value 0.812), LH (P-value 0.101), E2 (*P*-value 0.309), and total testosterone (*P*-value 0.633). There was a significant difference between the three groups regarding serum prolactin level (*P*-value < 0.001) (Table 2).

Semen analysis showed non-significant difference in the semen volume between control (3.06 ± 0.56 ml), azoospermia (2.53 ± 1.31 ml), and oligozospermia (2.97 ± 1.15 ml) groups (*P*-value 0.199). The total sperm concentration was lower in the oligozospermia group (51.53 ± 7.08) compared to the control group (59.39 ± 12.71) with no significant differences (*P*-value 0.352). The total and progressive motility were significantly lower in oligozospermia group (39.70 ± 16.63 and 20.11 ± 11.67) compared to the control group (52.06 ± 7.80 and 36.71 ± 2.52) (*P*-value < 0.001). The percent of abnormal forms was 92.00 ± 1.43 in control group and 91.00 ± 7.86 in oligozospermia group. No significant difference was observed between both groups regarding abnormal forms (*P*-value 0.467). DNA fragmentation was significantly higher among oligozospermia group (24.00 ± 10.99) compared to control group (16.77 ± 5.00) (*P*-value 0.001) (Table 2).

3.4 Bisphenol A, miRNA-337, and MDA results

The level of BPA was higher in azoospermia group (2.62 ± 0.71) compared to the oligozospermia group (0.96 ± 0.23) and the control group (1.37 ± 0.79) with significant differences between the three groups (*P*-value < 0.001) (Table 2).

The miRNA-337 gene expression was significantly elevated in oligozospermia group (2.70 ± 0.29) and

Table 2 Laboratory investigations of the studied groups

Items	Control (no = 35)	Azo (no = 16)	Oligo (no = 22)	*P*-value
FSH	10.4 ± 1.5	11.2 ± 5.3	11.2 ± 7.4	0.812
LH	15.7 ± 5.2	12.4 ± 4.8	13.7 ± 5.7	0.101
E2	25.3 ± 9.2	31.1 ± 17.7	26.2 ± 12.5	0.309
Total testosterone	5.5 ± 1.57214	6.2 ± 3.42	5.6 ± 2.8	0.633
Prolactin	13.9 ± 4.9a	8.2 ± 3.6b	11.0 ± 4.6a	< 0.001*
Semen analysis				
Volume	3.06 ± 0.56	2.53 ± 1.31	2.97 ± 1.15	0.199
Conc	19.43 ± 2.00	–	17.88 ± 14.87	0.545
Total conc	59.39 ± 12.71	–	51.53 ± 7.08	0.352
Total motility	52.06 ± 7.80a	–	39.70 ± 16.63b	< 0.001*
Progressive mot	36.71 ± 2.52a	–	20.11 ± 11.67b	< 0.001*
Normal morph	8.00 ± 1.43	–	8.54 ± 7.62	0.682
Abnormal forms	92.00 ± 1.43		91.00 ± 7.86	0.467
Immotile	47.94 ± 7.80a		60.29 ± 16.63b	< 0.001*
DNA frag	16.77 ± 5.00a		24.00 ± 10.99b	0.001*
Round cells		0(0.0%)	1(4.5%)	0.117
0–1/HPF	0(0.0%)	13(81.3%)	14(63.6%)	
0–5/HPF	35(100.0%)	1(6.3%)	1(4.5%)	
2–3/HPF	0(0.0%)	0(0.0%)	1(4.5%)	
2–4/HPF	0(0.0%)	0(0.0%)	1(4.5%)	
25–30/HPF	0(0.0%)	2(12.5%)	3(13.6%)	
3–5/HPF	0(0.0%)	0(0.0%)	1(4.5%)	
4–6/HPF	0(0.0%)			0.117
Bisphenol A	1.37 ± 0.79a	2.62 ± 0.71b	0.96 ± 0.23a	< 0.001*
mi-337	1.18 ± 0.14a	2.61 ± 0.29b	2.70 ± 0.29b	< 0.001*
MDA	0.94 ± 0.13a	1.64 ± 0.30b	1.55 ± 0.24b	< 0.001*

P*-value ≤ **0.05 is considered significant by (Chi-square test)

azoospermia group (2.61 ± 0.29) compared to the control group (1.18 ± 0.14) (*P*-value < 0.001) (Table 2).

The MDA level was significantly elevated in oligozospermia group (1.55 ± 0.24) and azoospermia group (1.64 ± 0.30) compared to the control group (0.94 ± 0.13) (*P*-value < 0.001) (Table 2).

3.5 The role of Bisphenol A, miRNA-337, and MDA in prediction of oliozospermia and azoospermia

The ROC curve analysis showed that at a cutoff > 1.11, the MDA could predict azoospermia and oligospermia with sensitivity (100%), specificity (100%), PPV (100%), and NPV (100%). At a cutoff > 1.7, the miRNA-337 could predict azoospermia and oligospermia with sensitivity (100%), specificity (100%), PPV (100%), and NPV (100%). At a cutoff > 1.49, the BPA could predict azoospermia with sensitivity (93.75%), specificity (80%), PPV (68.2%), and NPV (96.6%). At a cutoff ≤ 1.01, the BPA could predict oligozospermia with sensitivity (86.36%), specificity (54.29%), PPV (54.3%), and NPV (86.4%) (Tables 3 and 4).

4 Discussion

Approximately 10–15% of reproductive-aged men have male infertility, making it a common problem [9]. Male infertility may occur if there is a disruption to the complex and organized process of spermatogenesis. Various cell types in the testis integrate, communicate, and operate properly during germ cell development to ensure male fertility [10] (Table 5).

This study aimed to investigate the relation between infertility of adult Egyptian males and their urinary BPA, MDA, and miRNA-337 levels.

Our study found that the miRNA-337 gene expression was significantly elevated in oligozospermia group (2.70 ± 0.29) and azoospermia group (2.61 ± 0.29) compared to the control group (1.18 ± 0.14). Furthermore, we investigated the role of miRNA-337 gene expression in the prediction of azoospermia and oligozospermia. The ROC curve analysis showed that at a cutoff > 1.7, the miRNA-337 could predict azoospermia and oligospermia with sensitivity (100%) and specificity (100%).

Table 3 Pearson's correlation coefficients of Bisphenol A (BPA) levels with semen quality parameters, hormonal profile, and miRNA-337

Variable	Bisphenol A (BPA)					
	Control		Oligospermia		Azoospermia	
	r	*P*-value	*r*	*P*-value	*r*	*P*-value
Sperm concentration	−0.222	0.2	−0.094	0.667	–	–
Total sperm concentration	−0.037	0.833	−0.019	0.932	–	–
Total motility	0.080	0.648	0.111	0.623	–	–
Progressive motility	0.037	0.833	−0.014	0.951	–	–
Normal morphology	−0.088	0.614	−0.270	0.224	–	–
Abnormal forms	0.088	0.614	0.270	0.224	–	–
Immotile sperms	−0.080	0.648	−0.111	0.623	–	–
Testosterone	−0.139	0.427	−0.062	0.786	−0.489	0.055
Prolactin	−0.027	0.880	0.023	0.919	0.004	0.990
FSH	−0.116	0.505	−0.225	0.313	0.040	0.884
LH	−0.054	0.757	−0.183	0.416	0.305	0.251
E2	−0.028	0.874	0.170	0.450	−0.178	0.511
miRNA-337	0.089	0.611	0.294	0.183	−0.279	0.295

r = Pearson's correlation coefficient

Table 4 Sensitivity, specificity, PPV, NPV of miRNA-337, Bisphenol A, and MDA in prediction of azoospermia

	MiRNA-337	Bisphenol A	MDA
Cutoff	> 1.7	> 1.49	> 1.11
AUC	1.000	0.882	1.000
Sensitivity (95%CI)	100(79.4–100)	93.75(69.8–99.8)	100(79.4–100)
Specificity (95%CI)	100(90–100)	80.0(63.1–91.6)	100(90–100)
PPV (95%CI)	100(85–100)	68.2(52.2–80.8)	100(85–100)
NPV (95%CI)	100(90–100)	96.6(80.6–99.5)	100(90–100)

Table 5 Sensitivity, specificity, PPV, NPV of miRNA-337, Bisphenol A, and MDA in prediction of oligospermia

	MiRNA-337	Bisphenol A	MDA
Cutoff	> 1.7	≤ 1.01	> 1.11
AUC	1.000	0.671	1.000
Sensitivity (95%CI)	100(784.6–100)	86.36(65.1–97.1)	100(84.6–100)
Specificity (95%CI)	100(90–100)	54.29(36.6–71.2)	100(90–100)
PPV (95%CI)	100(85–100)	54.3(44.4–63.9)	100(85–100)
NPV (95%CI)	100(90–100)	86.4(67.9–95.0)	100(90–100)

To our knowledge, no previous studies investigated the role of miRNA-337 in cases of infertility. However, our results could be supported by the findings of Wang et al. [11] who investigated the role of miR-337 in androgen-dependent human cancer prostate and found that miR-337-3p mainly promotes apoptosis and cell growth inhibition under androgen deprivation in castrated animals in vivo and in vitro by simulating clinical antiandrogen therapies.

Moraveji et al. [12] reported that inhibiting the TGFb pathway leads to enhanced propagation of undifferentiated spermatogonia in vitro and in vivo and accelerates the recovery of spermatogenesis. Zhong et al. [13] revealed that miR-337 was very low and even hardly detected in the femoral head of adult rats, but highly expressed during the cartilage proliferation. The miR-337 was a supressor for TGFBR2. Transforming growth factor-β (TGF-β) signaling pathway plays important roles in regulating cell proliferation, differentiation, migration, and apoptosis in a broad spectrum of tissues.

However, variable results were obtained when investigating the role of miRNA-337 in rapidly proliferating cells like tumor cells. Tian et al. [14] results were in line with our findings as they concluded that microRNA-337-5p is upregulated in osteosarcoma tissues, which is an independent prognostic factor in osteosarcoma. They revealed that overexpressed microRNA-337-5p can promote proliferative and invasive abilities of osteosarcoma.

Unlike our study several reports found that miRNA-337 was significantly downregulating in rapidly dividing cells. In the study of Dong et al. [15], miR-337 was significantly downregulated in both cervical cancer tissues and cell lines. The low-expression level of miR-337 was correlated with tumor size. Cui et al. [16] found that miR-337 is underexpressed in HCC tissues and cell lines, and its reduced expression is correlated with TNM stage and lymph node metastasis. The difference could be

attributed to the different nature of tissues and the mechanism of proliferation in each cell type.

Assessment of BPA in our study showed that the level of BPA was significantly higher in azoospermia group (2.62 ± 0.71) compared to the oligozospermia group (0.96 ± 0.23) and the control group (1.37 ± 0.79) while no significant differences was observed between oligozospermia and control groups. The ROC curve analysis showed that at a cutoff > 1.49, the BPA could predict azoospermia with high sensitivity (93.75%) and specificity (80%). However, the role of BPA to predict oligozospermia at a cutoff ≤ 1.01 showed a high sensitivity (86.36%) and moderate specificity (54.29%).

Variable results were obtained regarding the relation between BPA and infertility. Mantzouki et al. [17] results were in line with our findings. In their study, they assessed the association between plasma BPA concentrations and semen quality in infertile men of specific etiology. They found no difference in serum BPA concentrations between infertile and fertile men, but they observed very high concentrations of BPA only in men with azoospermia.

This study found that there was no significant association between the level of BPA and different semen parameters. This was unlike the study of Chen et al. [18] who revealed in their study about the associations between urinary Bisphenol A and its analogs and semen quality that higher BPA and its substitutes BPS exposures were individually related to declined semen quality suggesting the potential reproductive health hazards in relation to BPA analogs and mixtures of bisphenols.

Also, the study of Shokry et al. [19] showed that there was a highly statistically significant negative correlation between sperm concentration and mean urinary BPA level ($P < 0.001$). Furthermore, there was statistically significant negative correlation concerning sperm motility and the mean BPA level ($P = 0.003$). However, there was no significant correlation concerning abnormal form of sperm and mean BPA level ($P = 0.178$). The divergent results are likely due to heterogeneity in the extent of BPA exposure, sample sizes, type of population, and enrollment setting.

The effect of BPA on reproductive system could be explained as BPA is commonly considered to have estrogenic and antiandrogenic effects able to disrupt the hypothalamic–pituitary–gonadal axis, and the ability to alter normal epigenetic patterns with impairing consequences on the reproductive system. BPA binding to estrogen receptors alters their ability to recruit tissue-specific coactivators important for differential tissue-dependent responses. In addition, it has been demonstrated that BPA has chemical affinity for a membrane-associated G protein-coupled estrogen receptor (GPER), equivalent to its primary ligand, estradiol. By binding to the GPER receptor, which expression has also been identified in the hypothalamus and pituitary [20].

The level of lipid peroxide in the blood was estimated by measuring the amount of malondialdehyde as a final product of oxidized fats. Among our studied patients, the MDA level was significantly elevated in oligozospermia group (1.55 ± 0.24) and azoospermia group (1.64 ± 0.30) compared to the control group (0.94 ± 0.13). The ROC curve analysis showed that at a cutoff > 1.11, the MDA could predict azoospermia and oligospermia with sensitivity (100%), specificity (100%), PPV (100%), and NPV (100%).

Our findings were consistent with the study of Agha et al. [21] who reported that the level of MDA was significantly higher in oligozospermia group (3.31 ± 0.15) compared to the control group (1.41 ± 0.99) (P-value 0.0001).

Also, Yusuf & Emokpae [22] showed that the mean levels MDA in normospermia, oligozoospermia, and azoospermia groups were significantly higher ($P = 0.01$) in the studied participants than the control group. The mean levels of and MDA were higher among azoospermia than oligospermia and least in the normospermia. Krzyściak et al. [23] showed in their study that MDA in seminal plasma of infertile patients was significantly higher compared to the control group.

It is well known that a small amount of ROS is vital for the steps involved in the essential physiological response of fertilization—sperm maturation, hyperactivation, capacitation, acrosome reaction of sperm, and sperm-oocyte fusion. However, lipid peroxidation (LPO) within the cellular membrane, deoxyribonucleic acid (DNA) fragmentation in nuclei and mitochondria, and apoptosis can occur when the production level of ROS gets excessive. All these events negatively affect sperm parameters, male fertility, and pregnancy outcome of their partners. Takeshima et al., 2021 [24].

5 Conclusion

While there is no significant change in the levels of Bisphenol A between normal fertile males and infertile males with oligozospermia, a significant elevation in the BPA level was observed in infertile males with azoospermia. A significant upregulation of the miRNA-337 gene expression in infertile males either oligozospermia or azoospermia was also observed. In addition, lipid peroxidation as evident by the significant elevation of MDA levels was marked among infertile patients.

Abbreviations

BPA	Bisphenol A
MDA	Malondialdehyde
miRNA-337	MicroRNA-337

Author contributions
MAA helped in arrangement of the practical part of the research and searching. NNI helped in arrangement of the practical part of the research and searching. SSG helped in organization of the teamwork, management of the research, supervision, and reviewing. DAEH helped in arrangement of the practical part of the research, searching, writing the research, and paperwork. RSAF helped in arrangement of the practical part of the research and searching. MMG helped in organization of the teamwork, supervision, and reviewing. AMA helped in arrangement of the practical part of the research, searching, writing the research, and paperwork. AML helped in arrangement of the practical part of the research and searching. AFMA helped in organization of the teamwork and management of the research.

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Availability of data and materials
Available.

Declarations

Ethical approval and consent to participate
All experimental protocols were approved by the Local Research ethical Committee for the care and use of laboratory animals present in Beni-Suef University. All methods were carried out in accordance with relevant guidelines and regulations. All methods are reported in accordance with ARRIVE guidelines (hppt://arriveguidelines.org) for the reporting of animal experiments.

Consent for publication
All authors have critically reviewed and approved the final draft and are responsible for the content and similarity index of the manuscript.

Competing interests
We have no conflict of interest to declare.

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