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Impact of tobacco smoking in association with H2BFWT, PRM1 and PRM2 genes variants on male infertility

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Abstract

Tobacco's genotoxic components can cause a wide range of gene defects in spermatozoa such as single- or double-strand DNA breaks, cross-links, DNA-adducts, higher frequencies of aneuploidy and chromosomal abnormalities. The aim in this study was to determine the correlation between sperm quality determined by standard parameters, sperm DNA maturity tested by Chromomycin A3 (CMA3) staining, sperm DNA fragmentation tested by TUNEL assay and tobacco smoking in association with the single nucleotides polymorphisms (SNP) of three nuclear protein genes in spermatozoa (*H2BFWT*, *PRM1* and *PRM2*). In this study, semen samples of 167 male patients were collected and divided into 54 non-smokers and 113 smokers. The target sequences in the extracted sperm DNA were amplified by PCR followed by Sanger sequencing. The results showed the presence of three variants: rs7885967, rs553509 and rs578953 in *H2BFWT* gene in the study population. Only one variant rs737008 was detected in *PRM1* gene, and three variants were detected in the *PRM2* gene: rs2070923, rs1646022 and rs424908. No significant association was observed between the concentration, progressive motility, morphology and the occurrence of *H2BFWT*, *PRM1* and *PRM2* SNPs. However, sperm parameters were significantly lower in heavy smokers compared to controls ($p < 0.01$) (sperm count: 46.00 vs. 78.50 mill/ml, progressive motility: 15.00% vs. 22.00%, and morphology 4.00% vs. 5.00%, respectively). Moreover, the heavy smoker individuals exhibited a considerable increase in CMA3 positivity and sDF compared to non-smokers ($p < 0.01$) (29.50% vs. 20.50% and 24.50% vs. 12.00%, respectively). In conclusion, smoking altered sperm parameters and sperm DNA integrity, but did not show a linkage with genetic variants in *H2BFWT*, and protamine genes (*PRM1* and *PRM2*).

KEYWORDS
H2BFWT, male infertility, *PRM1*, SNPs, tobacco smoking

1 | INTRODUCTION

During the last stages of spermatogenesis, the spermatozoa develop to have a highly organized genome. The sperm DNA is compacted by nearly 85% of protamines and 15% of histones carrying epigenetic signals, together with different kind of RNA molecules and proteins participate in post-fertilization events and mainly in embryo development.

Infertility or subfertility is a consequence of many pathological factors. In general, 50% of infertility instances are attributed to idiopathic subfertility. About 15% of these instances are associated to genes defects, involving chromosomal microdeletions, aneuploidy, Karyotype abnormalities (Harton & Tempest, 2012), variable number of tandem repeats (VNTR), gene copy number variations (CNVs). Moreover, single nucleotide polymorphisms (SNPs) in endocrine-function genes like *ESR1* and *ESR2*, specific spermatogenesis-function genes like protamine genes and *H2BFWT*, or common cell-function genes like *CYP1A1* and *MTHFR* (Krausz et al., 2015).

In other idiopathic male infertility cases, environmental and lifestyle factors, such as nutrition, drinking alcohol, physical activity and tobacco smoke, play an important role in the aggravation of infertility problems.

Contradictory findings have in fact been reported concerning the influence of tobacco smoke on standard semen parameters. Some studies have found that smoking is correlated with a decline in semen volume, count, motility and morphology (Al-Turki, 2015; Hamad et al., 2014; Hammadeh et al., 2010; Zhang et al., 2013) but others have not found this association (Davar et al., 2012; Kumar et al., 2014; Moretti et al., 2014).

In the last two decades, various research has been done on to explain how the behavioural and environmental factors, particularly smoking, affect the sperm genome and epigenome (Harlev et al., 2015) as well as their potentially impact on developing embryos (Beal et al., 2017; Donkin & Barrès, 2018).

Smoke causes oxidative DNA damage due to high amounts of seminal reactive oxygen species (ROS) like free radicals (superoxide (O_2^-), hydroxyl (OH), etc) and non-radical species like hydrogen peroxide (H_2O_2) (Hammadeh et al., 2010; Kumar et al., 2015; La Maestra et al., 2015; Opuwari & Henkel, 2016). Furthermore, cigarette smoke components have been associated with the production of DNA adducts leading to sperm DNA damage (Perrin et al., 2011; Phillips & Venitt, 2012). Moreover, the exchange between histone-protamine during spermiogenesis is also important because an incomplete protamination will expose the sperm DNA to an elevated risk of oxidative attack, which will damage it (Giwercman & Spanó, 2018). A correlation has been shown between tobacco smoke, altered spermatogenesis and variations in protein levels, such as protamine (protamine ratio) (Hamad et al., 2017; Hammadeh et al., 2010) and the protamine-to-histone ratio (Hamad et al., 2014; Yu et al., 2014).

In a study in humans, some researchers showed that smoking men had a four-fold higher possibility of passing on mutations (so-called tandem repeat minisatellites) to offspring (Linschooten et al., 2013).

Moreover, one research found that male smokers' spermatozoa have an elevated prevalence of disomy in the XY chromosomes and chromosome 3 (Pereira et al., 2014). In addition, developing amniocytes in a nicotine-containing environment resulted in an aberrant shape and number of 22, 21, 20, 15 and 8 chromosomes (Demirhan et al., 2011).

Besides, several studies have shown correlations between genetic variants of exogenous metabolism, such as *N*-acetyltransferase (NAT2), cytochrome P450 (CYP1A1) (Yarosh et al., 2013), glutathione-*S*-transferase (GSTs) and idiopathic infertility in smokers (Yarosh et al., 2015).

Our goal was to demonstrate the effect of smoke in association with certain paternal genome variants on spermatozoa structure like DNA and its function. Specifically, we aimed to sequence the H2B histone family member W, testis-specific gene: *H2BFWT* and the protamine: *PRM1* and *PRM2* genes, to find out possible associations with sperm parameters, sperm DNA fragmentation and protamination.

2 | MATERIAL AND METHODS

2.1 | Samples gathering and preparation

Semen samples ($n = 167$) were collected randomly from male partners of couples undergoing ICSI treatment. Each patient had a physical examination and was interviewed following an organized questionnaire (Medical history, job, lifestyle and tobacco smoke). The participant who smokes more than one pack/day for 10 years or 2 pack/day for 5 years was considered as heavy-smoker, and the participant who did not smoke was considered as non-smoker.

Patients were divided later to 54 proven fertile non-smoker males 'as controls' and 113 samples obtained from heavy smoker males 'as cases'.

All the samples were obtained depending on the following exemption criteria: age over 45 years, diabetes mellitus, alcohol drinkers, and the presence of anti-sperm antibodies, varicocele, and Y chromosome microdeletions. Besides, the female partners should have no female-related cause of subfertility.

Briefly, semen parameters were evaluated based on WHO laboratory guidelines (World Health Organization, 2010). Before the isolation step of DNA, samples were loaded on a 45%–90% gradient (Nidacon International) to purify them from the somatic cells and other debris. The local ethics committee approved the study [195/11]. Prior to inclusion in this study, all participants gave written informed consent.

2.2 | Detection of variants in *H2BFWT*, *PRM1* and *PRM2* genes

The Isolate II DNA/RNA/Protein Kit (Bioline, UK) was used to extract genomic DNA from purified samples. Then, the amount and purity of DNA was evaluated utilizing a Nanodrop spectrophotometer

ND-2000c (Thermo Scientific, USA) and kept at −80°C. Studied genes were amplified using conventional PCR methods. Primer3 was used to design primers (F: forward and R: reverse), which depended on reference sequencing of three genes from GenBank (Untergasser et al., 2012).

Gene	Primer
H2BFWT	*F*: TGGCATGGATCAGCTGAGAA
	R: GGACACTCCCTAAGCCTACT
PRM1	*F*: CCTTTGCCCTCACAATGACC
	R: AACAAAACCCAGCGTGACAA
PRM2	*F*: CCAACAGTAACACCAAGGGC
	R: GCCAGGTTTGTGTGATTCGT

The polymerase chain reaction (PCR) was made utilizing MyTaq™HS Red Mix Kit (Bioline, UK) and the C1000™ Thermal cycler, Bio-Rad, USA. Then, 5 µl of each amplified sample for each gene was examined by agarose gel electrophoresis (Biolabs, USA). Then, Qiagen Miniprep PCR-purification HT was used to purify the rest of PCR products. Finally, Sanger sequencing method, and two Single Read HTs (Qiagen, Germany) were constructed for all genes.

2.3 | Assessment of sperm DNA

The Chromomycin A3 (CMA3) stain was utilized to assess the protamine insufficiency (Manicardi et al., 1995) and the TUNEL (terminal deoxyribonucleotidyl transferase-mediated dUTP nick-end labelling) test was performed to determine the fragmentation in the DNA of sperm (Borini et al., 2006).

For the first stain, the first step was the fixation of the slides for 1 h using acetic acid-methanol. After that, slides were stained with CMA3 solution for 30 min and kept in the dark at room temperature (RT). Slides were mounted and stored overnight at 4°C after being washed in phosphate buffer saline (PBS).

For the TUNEL assay, various steps were performed according to the instructions for the Fluorescein in Situ Cell Death Detection Kit (Roche Diagnostics, Germany).

After staining, 500 spermatozoa per slide (Olympus, Japan) were examined utilizing a fluorescence microscope. Sperm were grouped according to CMA3 staining as follows: dark green sperm (CMA3 negative) and light green sperm (CMA3 positive) (Amor, Shelko, et al., 2019). Sperm are classified according to the TUNEL test like this: blue sperm (TUNEL negative) and green sperm (TUNEL positive) (Amor, Shelko, et al., 2019).

2.4 | Statistical evaluation

IBM SPSS version 24.0 (SPSS Inc., USA) was employed for statistical analysis. The samples were found not to be normally distributed. Mann–Whitney *U* tests were used to compare quantitative factors, and Spearman analyses were performed to determine associations between the different parameters.

Allele frequencies for each gene were performed utilizing the Tracy tool (https://github.com/gear-genomics/tracy). Variant calling was performed using aligner bwa (Li & Durbin, 2009), WhatsHap (Ebler et al., 2018; Patterson et al., 2015), and samtools mpileup (Li et al., 2009) (Figure 1).

From all SNPs generated by the previous procedure, all regions with an allelic distribution greater than 5% among all examined participants were selected and HWE was scored using Fisher's exact test. Moreover, Fisher's exact test was applied to verify associations of allelic frequencies between the groups of non-smokers and heavy smokers. Then, Benjamini-Hochberg correction was used to correct multiple testing ($\alpha = 0.05$).

3 | RESULTS

3.1 | Study population and semen parameters

One hundred and sixty-seven samples were entered in this study. Fifty-four from proven fertile non-smoker males 'as controls' and 113 samples obtained from heavy smoker males 'as cases' (Table 1). Compared with controls, sperm count, progressive motility, and normal morphology were reduced significantly in the case group ($p \leq 0.009$, $p \leq 0.001$, $p \leq 0.002$, respectively). In contrast, the percentage of chromomycin A3 positivity and sDF showed a significant increase in the case group compared with the control group ($p \leq 0.003$ and $p \leq 0.0001$, respectively).

3.2 | Quality control

All sites with an allele frequency of more than 5% throughout all examined participants were chosen from all resulting SNPs from the preceding steps. The outcome was a set of nine SNP regions. On chromosome X, three of them were recognized. Since all sequenced participants were males, each of these regions should be genotyped as either 0/0 or 1/1 because each male has just one X chromosome. However, in all participants, all SNPs on the X chromosome were genotyped. On chromosome 16, the remaining six SNPs were identified. Fisher's Exact Test was applied to verify whether they were in Hardy–Weinberg Equilibrium (HWE). Four of the six SNPs did not significantly differ from HWE. We found that the two other SNPs whereby the analysis revealed a significant difference from HWE may not be actual variations and ruled them out of further investigation. As a result, seven SNPs were identified from our last group of SNP calls.

Additionally, we found that the 1000 genomes project reported our SNP calls (1000 Genomes Project Consortium, 2012). As a further quality control, we compared the distribution of alleles identified by these SNPs with the distribution of alleles from 1000 genomes and observed that they corresponded reasonably well. Figure 2 Shows the results. The blue dots represent the observed allele frequencies in our sample, while the boxplots represent the reported allele frequency

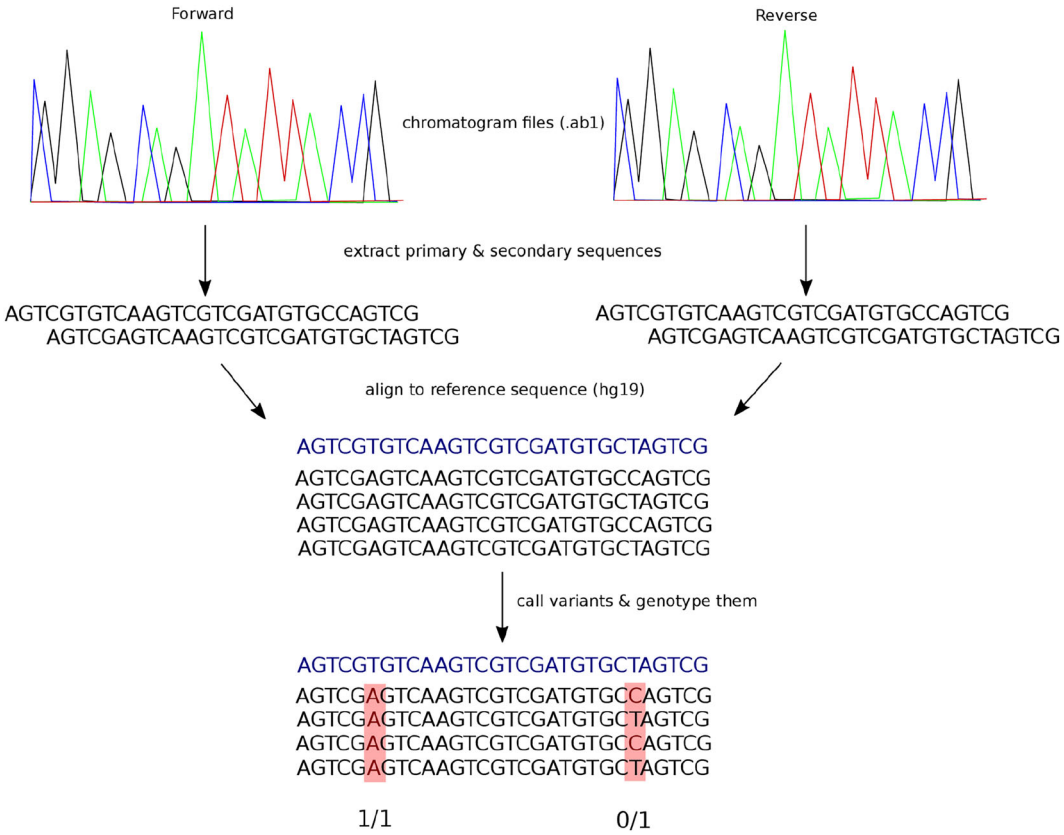

FIGURE 1 Variant calling. Allelic sequences were extracted from the chromatogram files and aligned to a reference genome. Next, variants were called and genotyped in all samples

TABLE 1 Compared parameters between control group (non-smokers) and case groups (heavy smokers)

Parameters	Non-smokers ($n = 54$)	Heavy smokers ($n = 113$)	p Value
Semen volume (ml)	3.00 (1.2–8.3)	3.00 (0.7–9.0)	0.325
Sperm count (10^6 per ml)	78.50 (9–286)	46.00 (1–197)	0.009**
Progressive motility (PR) (%)	22.00 (0–80)	15.00 (0–50)	0.001**
Normal form (%)	5.00 (0–53)	4.00 (0–13)	0.002**
CMA3 positivity (%)	20.50 (0–63)	29.50 (0–98)	0.003**
Sperm DNA fragmentation (sDF) (%)	12.00 (2–60)	24.50 (0–97)	0.0001**

Note: Results are expressed as median values and interquartile range (25th–75th percentile).
**p Value is statistically high significant at the 0.01 level.

distributions for these variants in the different studied populations by the 1000 Genomes project.

3.3 | H2BFWT, PRM1 and PRM2 SNPs distribution between non-smokers and heavy smokers

A table was constructed for every SNP of non-smoker and heavy smoker groups by calculating the reference and alternative alleles number throughout the participants in each group. The purpose was to assess the correlation between alleles of SNP and each class. Then, Fisher's Exact Test was used to determine considerable variations in allele frequencies between the two groups and a Benjamini Hochberg correction ($\alpha = 0.05$) was utilized to correct for multiple experiments. Neither of the SNPs was found to be significant. Table 2 shows all investigated SNPs as well as their allele frequencies.

3.3.1 | The SNPs of H2BFWT gene

Since all our participants are male, the genetic variants, rs578953, rs553509 and rs7885967 were shown to be homozygous. Allelic distribution was not different between non-smokers heavy smokers. The rs553509 was found in exon 1 and was classified as a missense variant converting arginine to histidine. The rs7885967 is in the 5′-untranslated region.

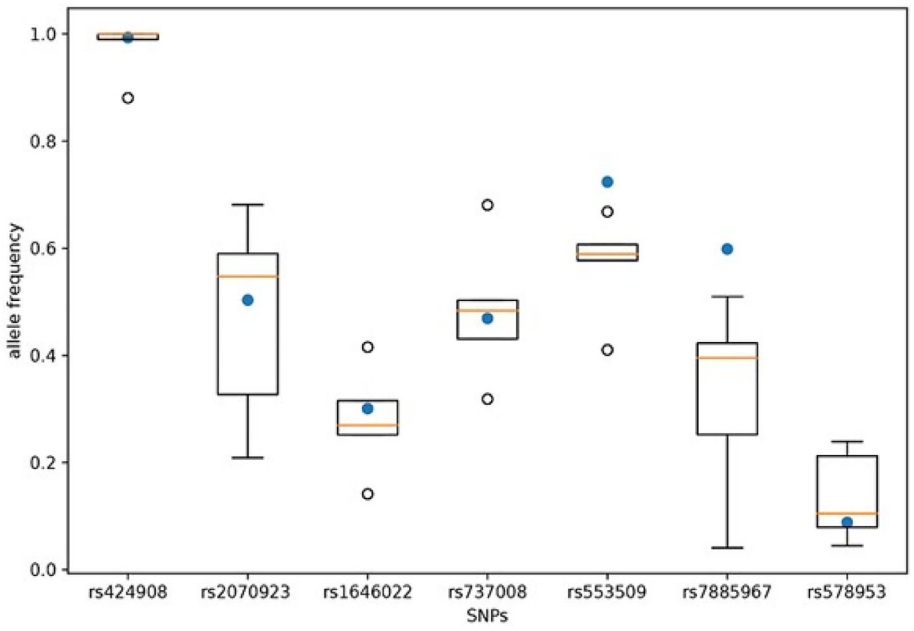

FIGURE 2 Comparison to 1000 genomes allele frequencies. We compared the allele frequencies for our detected SNPs (blue) to the ones previously reported by the 1000 genomes project (1000 Genomes Project Consortium, 2012) for these variants across several population

TABLE 2 Detected SNPs and their allele frequencies

Genomic position (hg19)	ID	Reference allele	Alternative allele	Allele frequency (across all samples)
Chromosome 16 11374866	rs737008	G	T	0.47
Chromosome 16 11369534	rs424908	G	A	0.99
Chromosome 16 11369855	rs2070923	G	T	0.50
Chromosome 16 11369930	rs1646022	C	G	0.30
Chromosome X 103267865	rs553509	C	T	0.72
Chromosome X 103268241	rs7885967	G	A	0.60
Chromosome X 103268333	rs578953	G	A	0.09

TABLE 3 Recapitulation of results obtained by direct sequencing of PCR products including the H2BFWT gene (genomic and allelic frequencies are denoted)

			Heavy-smokers		Non-smokers		*p* Value
SNP	Gene region	AA change	Genotype	Allele	Genotype	Allele	
chrX g.103268241G > A	5 prime UTR	NA	A/A (65) 0.59 G/A (0) 0.0 G/G (46) 0.41	A = 0.59 G = 0.41	A/A (20) 0.63 G/A (0) 0.0 G/G (12) 0.37	A = 0.63 G = 0.37	NS
chrX g.103267865C > T	Exon 1	R/H	T/T (77) 0.7 C/T (0) 0.0 C/C (33) 0.3	T = 0.7 C = 0.3	T/T (25) 0.81 C/T (0) 0.0 C/C (6) 0.19	T = 0.81 C = 0.19	NS
chrX g.103268333G > A	upstream	NA	A/A (8) 0.07 G/A (0) 0.0 G/G (103) 0.93	A = 0.07 G = 0.93	A/A (5) 0.16 G/A (0) 0.0 G/G (27) 0.84	A = 0.16 G = 0.84	NS

Abbreviations: AA, amino acid; NA, not applicable; NS, no significant differentiation was distinguished by comparing the genotype and allele frequencies between the heavy-smoker and non-smoker groups; UTR, untranslatedregion.

In Table 2, the distribution of alternative alleles for these two SNPs was larger than the reference allele. Therefore, the numbers of non-smokers and heavy smokers with homozygous minor types were greater than those with homozygous major types (Table 3).

For the rs578953 SNP, which is an upstream variant, the frequency of the reference allele is greater than the frequency of alternative allele (0.09). Here, five non-smokers and eight heavy smokers were homozygous minor type (A/A), while twenty-seven non-smokers and one hundred and three were homozygous major type (G/G).

3.3.2 | The SNPs of PRM1 gene

Among our study participants, only the SNP rs737008 was reported in PRM1 gene. It is located at position g.11374866G > T of the PRM1 coding region (exon 2) and is a synonymous variant. Allele frequencies did not differ between non-smokers and heavy smokers (Table 4). Fifteen non-smokers and 32 heavy smokers were homozygous for major type G/G, 7 non-smokers and 30 heavy smokers were heterozygous (G/T), and 11 non-smokers and 46 heavy smokers were heterozygous (G/T). Smokers were homozygous for the minor type (T/T) (Table 4).

3.3.3 | The SNPs of PRM2 gene

Three SNPs were identified in PRM2 gene, as shown in Table 5. Two variants: rs2070923 and rs1646022 were found in the intergenic region, and rs424908 was found in the 3′-untranslated regions. By comparing the allelic distribution between non-smokers and heavy smokers, it did not differ significantly. For the first variation, four non-

TABLE 4 Recapitulation of results obtained by direct sequencing of PCR products including the PRM1 gene (genomic and allelic frequencies are denoted)

			Heavy-smokers		Non-smokers		
SNP	**Gene region**	**AA change**	**Genotype**	**Allele**	**Genotype**	**Allele**	***p* Value**
chr16 g.11374866G > T	Exon 2	None	T/T (30) 0.28 G/T (46) 0.43 G/G (32) 0.29	T = 0.49 G = 0.51	T/T (7) 0.21 G/T (11) 0.33 G/G (15) 0.46	T = 0.38 G = 0.62	NS

Note: AA, amino acid; NS: no significant differentiation was distinguished by comparing the genotype and allele frequencies between the heavy-smoker and non-smoker groups.

TABLE 5 Recapitulation of results obtained by direct sequencing of PCR products including the PRM2 gene (genomic and allelic frequencies are denoted)

			Heavy-smokers		Non-smokers		
SNP	**Gene region**	**AA change**	**Genotype**	**Allele**	**Genotype**	**Allele**	***p* Value**
chr16 g.11369930C > G	Intron	NA	G/G (15) 0.14 C/G (38) 0.34 C/C (58) 0.52	G = 0.31 C = 0.69	G/G (4) 0.13 C/G (9) 0.28 C/C (19) 0.59	G = 0.27 C = 0.73	NS
chr16 g.11369855G > T	Intron	NA	T/T (31) 0.28 G/T (47) 0.42 G/G (33) 0.3	T = 0.49 G = 0.51	T/T (12) 0.38 G/T (11) 0.34 G/G (9) 0.28	T = 0.54 G = 0.46	NS
chr16 g.11369534G > A	3 prime UTR	NA	A/A (110) 0.99 G/A (1) 0.1 G/G (0) 0.0	A = 1.0 G = 0.0	A/A (31) 0.97 G/A (1) 0.03 G/G (0) 0.0	A = 0.98 G = 0.02	NS

Abbreviations: AA, amino acid; NA, not applicable; NS, no significant differentiation was distinguished by comparing the genotype and allele frequencies between the heavy-smoker and non-smoker groups; UTR, untranslatedregion.

smokers and fifteen heavy smokers were homozygous for the minor type (G/G). Nineteen non-smokers and fifty-eight heavy smokers were homozygous for the major type (C/C).

The second intergenetic variation was also divided into three categories: Minor homozygotes (T/T) for 12 non-smokers and 31 heavy smokers, homozygotes major type (G/G) for 9 non-smokers and 33 heavy smokers, and heterozygous (G/T) for 11 non-smokers, and 47 heavy smokers. Furthermore, Table 2 showed that for each group examined, only one individual was heterozygous (G/a) and the rs424908 SNP was homozygous minor type (a/a), with an allelic distribution equal to 0.99 throughout all subjects.

3.4 | Correlation between detected variants and conventional spermiogram test parameters, sperm DNA fragmentation, and protamine deficiency

Wilcoxon rank-sum test was used for every integration of a variant and a phenotype. First, the frequency of values for subjects with genotype 0/1 and 1/1 to the frequency of values for subjects with genotype 0/0 was investigated for every SNP region and every spermiogram parameter. Following that, the frequencies for genotypes 0/0 and 0/1 to the frequencies for subjects with genotype 1/1 were compared. Then, using the Benjamini Hochberg correction ($\alpha = 0.05$), we corrected for multiple testing once again. There was no evidence for an association between any of the SNPs and the phenotypes.

4 | DISCUSSION

The genotoxic components of tobacco cause cross-linking of DNA adduct, single- or double-strand breaks, chromosomal abnormalities and aneuploidy, and other genetic changes in sperm (Beal et al., 2017).

Damage to sperm DNA might result in significant mutations if it is not repaired properly. These mutations can be passed down through generations, and the sorts of them range from single nucleotide variations (SNVs) and insertions/deletions (indels) to a wide spectrum of structural changes (Beal et al., 2017).

Benzo[a] pyrene and nicotine were shown to induce harmful changes of sperm DNA that can be passed down to children (Holloway et al., 2007; Mohamed et al., 2010). Furthermore, previous researchers have attributed genetic mutations or SNPs in enzyme genes involved in xenobiotic metabolism to infertility in smoker males, such as 4621Ile/Val in the cytochrome P450, family 1, subfamily A polypeptide 1 gene (CYP1A1) (Yarosh et al., 2013) and 590G > A variant in the N-acetyltransferase-2 gene (NAT2) (Yarosh et al., 2015).

Amor et al. (2021) showed that smoking negatively alters the sperm standard parameters, DNA stability of sperm and the ratio of protamine mRNA as well as downregulates the expression of *H2BFWT*, *PRM1* and *PRM2*. These alterations impair the sperm quality of the male (Amor et al., 2021).

In comparison to the control group, we have identified a significant reduction for these parameters in the case group ($p < 0.01$): sperm count, progressive motility, and normal morphology (46.00 mill/ml vs. 78.50 mill/ml, 15.00% vs. 22.00%, and 4.00% vs. 5.00%, respectively) as shown in Table 1. On the contrary, for the heavy smoker individuals, our findings have shown a significant increase ($p < 0.01$) in CMA3 positivity and sDF in comparison to non-smokers (29.50% vs. 20.50% and 24.50% vs. 12.00%, respectively) (Table 1).

These results are in accordance with other studies on the negative effects of smoking on sperm quality and its DNA structure (Amor et al., 2021; Amor, Nyaz, & Hammadeh, 2019; Hamad et al., 2014; Hammadeh et al., 2010). Sharma et al. (2016) noted in a meta-analysis that smoking negatively affected sperm parameters that were more pronounced in infertile than fertile individuals (Sharma et al., 2016). However, other investigations have found that tobacco has no impact on standard semen parameters (Martini et al., 2004; Sepaniak et al., 2006; Trummer et al., 2002). Thus, more molecular researches are needed to understand the mechanism of how tobacco smoking impacts male fecundity.

We investigated also potential relationships between genetic changes in the *H2BFWT*, *PRM1* and *PRM2* genes and subfertility. For *H2BFWT* (Xq22.2), 3 homozygous SNPs were identified: rs578953, rs553509 and rs7885967. But no difference in allele frequency was noticed between the non-smokers and heavy smokers (Table 3).

For the *PRM1* gene, one SNP has been found: rs737008 (Table 4). For the *PRM2* gene, 3 SNPs: rs2070923, rs424908 and rs1646022, were identified (Table 5). Similarly, for the protamines genes, no differences in allele frequency were identified between non-smokers and heavy smokers.

Moreover, the patients were divided into two groups, those who did have a variant and others who did not. The identified SNPs showed no influences on the standard sperm parameters and sperm DNA integrity tests.

To our knowledge, this is the first study to describe two *H2BFWT* gene variants in an ICSI patient population: rs424902 and rs57895.These results are agreeing with a study by Zargar that showed no correlation between two variants of the *H2BFWT* gene, rs7885967 and rs553509, and male subfertility (Haji Ebrahim Zargar, 2015). However, conflicting results identified an association between these two variants and men diagnosed with non-obstructive azoospermia and oligospermia (Lee et al., 2009). Same results were observed in other studies (Rafatmanesh et al., 2018; Ying et al., 2012).

Consistent with our findings, several studies have found no correlation between male subfertility and the *PRM1* variant rs737008 (Aoki et al., 2006; Imken et al., 2009; Jodar et al., 2011; Ravel et al., 2007; Tanaka et al., 2003; Venkatesh et al., 2011). However, Jiang et al. (2015) showed a substantial correlation between this genetic variant and male subfertility (Jiang et al., 2015).

Moreover, other studies have shown that two variants of the PRM2 gene, rs1646022 and rs2070923, are not associated with subfertility (Aoki et al., 2006; Imken et al., 2009; Jodar et al., 2011; Ravel et al., 2007; Tanaka et al., 2003; Venkatesh et al., 2011). However, Jiang et al. (2015) identified the variant rs1646022 as a hazard factor for male subfertility in an Asian subgroup (Jiang et al., 2015).

5 | CONCLUSION

The current research demonstrated the lack of a correlation between the identified variant alleles and each of non-smoker and heavy smoker classes. These findings indicate that tobacco smoking seems unlikely to alter the nucleotide sequence of these genes but may cause epigenetic alterations modifying the expression of the studied genes as demonstrated previously by (Amor et al., 2021).

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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