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Forensic Relationship between ATP6 Gene and Sperm Motility in a Saudi Population

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Abstract: Approximately 600 bp of ATP synthase subunit 6 mitochondrial gene (ATP6) were amplified and sequenced for 110 semen samples collected from infertile and normal Saudi men inhabiting Riyadh province. These data were analyzed in order to identify the Single Nucleotide Polymorphisms (SNPs) associated with male infertility. Six silent substitutions G8697A, G8856A, T8889C, T8952C, C9075T and C9060A were recorded with one novel site (G8856A) in the infertile men. Seven non- synonymous substitutions were also obtained with 4 novel sites (C8684T, G8860A, C8876T and G9055A) in the infertile men. Nine haplogroups (H2a, H1a, H5a, T, J2a, K1a, N1b, M25 and U7) were predicted. H5a, M25 and U7 were found in the oligoasthenoteratospermic (AOT) infertile men only with a percentage of 4.5%. It could be concluded that there was a correlation between ATP6 substitutions/haplogroups and male infertility since specific SNPs and haplogroups appeared in the AOT infertile men only. The present results are considered preliminary for using the mitogenome of the infertile men in a forensic perspective. More mtDNA data from infertile men are necessary to assess mtDNA forensic contributions in male fertility. Examination of autosomal Short Tandem Repeats (STRs) and Y chromosome -STR profiles for infertile men should be conducted to ascertain any changes in the genetic loci.

Keywords: Fertility, ATP6 Gene, Saudi Men, Haplogroups

Introduction

Infertility is a condition in the reproductive system characterized by the disability of pregnancy after twelve months or more of usual uncontrolled intercourse and there are nearly 15% of partners may have gone through it (Karimian and Hosseinzadeh Colagar, 2016). Approximately, 50% of infertility is probably caused by male factors. About 60% of reasons are unknown and nearly 30% are caused by genetic factors (Ferlin *et al*., 2006). Sperm motility is an important indicator for fertility (Eskenazi *et al*., 2003).

Several studies have suggested that sperm motility and male fertility can be reduced by mtDNA mutations (Baklouti-Gargouri *et al*., 2013; Zhou and Xie, 2017). Other investigations confirmed the correlation between male infertility and mtDNA deletions (Kumar *et al*., 2009; Hosseinzadeh Colagar and Karimi, 2014; Chari *et al*., 2015; Bahrehmand Namaghi and Vaziri, 2017). Talebi *et al*. (2018) recorded deletions of approximately 4977 and 7599 bp in the mtDNA of infertile men. These authors considered these deletions as a major risk factor for reduction in sperm motility and unsuccessful fertilization. Spiropoulos *et al*. (2002) found an association between A3243G mutation and sperm motility. Mughal *et al*. (2017) revealed multiple deletions in 7.8 kb fragment with high frequency in the infertile men. Heidari *et al*. (2016) recorded 10 base variants with 6 new substitutions and 4 variants in disorders other than infertility. Several mtDNA rearrangements have been reported in men with oligoasthenozoospermia (Lestienne *et al*., 1997). In ND4 gene, C11994T substitution was associated with asthenozoospermia in Indian males (Selvi Rani *et al*., 2006) however, Pereira *et al*. (2007) did not report such mutation in Portugal men. The non-synonymous T8821C mutation in ATP6 gene had been reported in severe oligoasthenoteratozoospermia but not in cases with oligospermia and normospermia (Holyoake *et al*., 2001). Significant nucleotide changes in ND2 (4769, 5400), ATP8 (8394), ATP6 (8701, 8860, 8879, 9098), ND3

(10165, 10172, 10207, 10398), ND4 (11719) and ND5 (12705, 13705, 13708, 13946) genes have been reported in infertile males (Kumar and Sangeetha, 2009). By comparing the complete mtDNA of 628 azoosepermic to that of 584 healthy Han Chinese men, 13 haplogroups and 10 susceptible variants were found (Ji *et al*., 2018) with M8a being associated with the non-obstructive azoospermia. This disease was diagnosed by a novel substitution at the position 8684C>T. Wu *et al.* (2019) revealed that sperm mtDNA copy number showed more diagnostic power than mtDNA deletions for the sperm abnormality. Ji *et al*. (2017) revealed that the variant 11696G>A exhibited significant higher frequency in idiopathic oligoasthenospermic Chinese men and this SNP was correlated with low sperm motility supporting the genetic susceptibility to oligoasthenospermia. Holyoake *et al.* (1999) found a polymorphic T to C transition in the ATP6 gene and suggested that the failure of immature spermatids having this mutation to fully develop.

In spite of the strong correlation between infertility and the mitogenome mutations, several studies did not support the correlation between the disease and the mitochondrial DNA variants (Pereira *et al*., 2005; Bandelt, 2008; Palanichamy and Zhang, 2011). Cummins *et al*. (1998) reported that there was no direct association between male infertility and the deletion of 4977 bp in the mtDNA. Ieremiadou and Rodakis (2009) detected 4977 bp deletions in males of varying ages with a negative association to sperm motility. Palanichamy and Zhang (2011) reported that mitogenome mutations except C8394T, C10165T, C10207T and G13708A were generally widespread in Indian men and could not be associated with male infertility.

The mtDNA haplogroups is constructed through accumulations of mutational changes in a population across time (van Oven and Kayser, 2009). Ruiz-Pesini *et al*. (2000) revealed that H haplogroup was represented with low percentage while T haplogroup is highly represented in men with low sperm motility. The sperm aberrations in shape, number and motility could be identified by specific mtDNA variants scattered in the mitogenes related to ATP production (i.e., ATP6 gene). These variants could be of a forensic value in identifying the men possessing infertility features. Refaat (2016) found a significant association of Y-STRs and azoospermia prevalence and suggested the use of Y-STR in azoospermia prognosis.

Investigating the contribution of mtDNA mutations in male infertility among Saudi populations is essential for the management of the disease. MtDNA is maternally inherited (Schon *et al*., 2012) and therefore mtDNA mutations that are associated with male infertility could serve as a biomarker for screening women that have high risk of transmitting such disease to their male children. The present study aimed to reveal the association between ATP6 genemutations and sperm motility in a Saudi population and to correlate sperm motility with haplogroups. We therefore amplified and sequenced ATP6 gene for different seminal samples with different sperm conditions for this purpose.

Materials and Methods

Sampling

A total of 110 semen samples were collected from infertile (66) and fertile (44) males at Thuriah Medical Centre in Riyadh, Saudi Arabia. The Institution Review Board approval was obtained (approval number 138367). Men ages from whom the samples were collected ranged between 21 and 70 years old. All the precautions of WHO (2010) guidelines for semen collection and transportation have been followed. The controls were normal men who had fathered children and have normal seminal and hormonal profiles. Men who have undergone vasectomy and other surgical operation in their reproductive system were excluded. Samples stayed in room temperature more than one hour were also excluded. Demographic data and other information were collected with a structured questionnaire.

Analysis of Semen Parameters

For semen collection, transportation and macroscopic examination, guidelines of WHO (2010) Laboratory Manual for the Examination and Processing of Human Semen, Fifth Edition were followed. The semen liquefaction was examined in 30 minutes after ejaculation. Men who had undergone vasectomy or any other surgical operation of their reproductive system were excluded. The samples that stayed at room temperature for more than one hour were also excluded. The colour of the semen was also examined and recorded. Using a graduated disposable pipette, the semen volume was determined. By spreading a drop of semen onto the pH paper, the pH was then measured, after which the developed colour was compared with the calibration strip within 30 seconds. The microscopic examination included sperm count, sperm morphology, percentage motility, viability, number of pus cell, red blood cells (RBCs) and epithelial cells. Sperm count was measured in millions of sperms per ml semen. The sperm abnormalities were oligozoospermia ($<15\times10^6$ spermatozoa/ml), asthenozoospermia (<40%) and teratozoospermia (<4% normal). Oligo-Astheno-Teratozoospermia (OAT) syndrome was found when the three conditions were found together. The collected samples contained all the above-mentioned abnormalities together, either in couples or separately. The controls (normozoospermia) exhibited normal sperm count, shape and motility.

DNA Extraction, PCR and Sequencing

Semen samples were taken from -80°C freezer and incubated in room temperature until thawed completely. Samples were centrifuged at 2000 rpm for 5 min. About 5 µL sperms in the bottom were transferred to 1.5 mL tube. 100 µL of buffer X (20 mM Tris Cl (pH 8.0), 20 mM EDTA, 200 mM NaCl and 4%SDS), 20 µL of proteinase k and 50 µL of DTT (Dithiothreitol) were added. The tube was vortexed and incubated at 56°C for 2 hours. Further extraction steps were conducted using Qiagen DNeasy blood and tissue kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol.

Polymerase Chain Reaction (PCR) was conducted in 40 µL reaction mixtures. The PCR tube contained 2 µL DNA template, 20 µL GoTaq Green Master Mix (Promega Corporation, Madison, WI 53711-5399, USA), 17.6 µL nuclease free water and 20 pmoles of each primer (forward primer: 5'- TCT GTT CGC TTC ATT CAT TG -3' and the reverse primer: 5'- TGA AAA CGT AGG CTT GGA-3'). The PCR conditions were adjusted according to Amer *et al.* (2013). Amplified ATP6 gene were examined for its integrity on 1% agarose gel electrophoresis and visualized under UV with ethidium bromide. Amplicons were purified using FavorPrep Gel Purification Mini Kit (Cat. No. FAGPK001) as described by the manufacturer. Purified products were subjected to sequencing from both strands using Macrogen facilities (https://dna.macrogen.com/eng/member/login.jsp?backURL=/eng/account/payment/payment_status.jsp).

Statistical Analysis

Data were treated with DNASIS v. 3.5 and MacClade V. 4.10 software (Maddison and Maddison 2002) for sequence alignment and was compared to the revised Cambridge Reference Sequence (Andrews *et al*., 1999). ATP6 sequences were deposited in the DDBJ/NCBI Genbank with their accession numbers (MN400447 - MN400556). ATP6 haploroups were predicted by using the online MitoMaster program using HaploGrep2 with Phylotree 17 for haplogroup determination (Topic revision: r3 - 02.May2017, MarieLott)https://www.mitomap.org/foswiki/bin/view/MITOMASTER/WebHome).

Results

Table 1 shows samples, which exhibited a combination of three abnormal semen parameters (OAT): low sperm concentration (oligoasthenoteratozoospermia), poor sperm motility (asthenozoospermia) and abnormal sperm morphology (teratozoospermia). Samples of this group suffered from primary infertility except samples 20, 62, 64 and 67, which were suffering from secondary infertility. In this group, sperm count did not exceed 13 million/ml semen and the total sperm motility did not exceed 33%. FSH, LH and prolactin hormones were high while testosterone was normal (data not shown). Table 2 listed the semen samples of OT, AT and T groups. OT group showed high FSH and LH hormones and normal testosterone hormone. AT group exhibited normal FSH and LH hormones and high prolactin hormone. T group showed normal FSH, LH, Testosterone and prolactin hormones (data not shown). Infertile group OT exhibited low sperm concentration (oligoasthenozoospermia) with abnormal sperm morphology (teratozoospermia) and normal sperm motility.

Table 1: OAT group with primary and secondary infertility

Sample no.	Sperms count. Mill/ml	Total motility%
1	0.80	25
2	3.00	10
3	7.00	20
4	5.00	2
5	1.20	33
6	0.10	1
7	0.26	15
8	0.60	1
9	7.00	25
10	7.00	10
11	0.20	1
12	11.00	27
13	3.00	16
14	1.80	25
15	6.00	20
16	0.20	2
17	3.00	20
18	3.00	20
19	7.00	20
20	0.30	9
21	8.00	7
22	0.30	10
23	8.00	37
24	0.80	4
25	7.00	14
26	0.50	20
27	3.00	5
28	0.20	2
29	9.00	33
30	5.00	26
31	0.50	8
32	10.00	30
33	12.00	25
34	7.00	11
35	0.50	2
36	3.00	8
37	6.00	25
38	6.00	10
39	0.10	2
40	1.00	2
41	9.00	11
42	1.80	16
43	13.00	23
44	1.00	2
45	5.00	8
46	2.00	15
47	1.80	20

Table 2: Distribution of OT, AT and T in the infertile group

Sample no.	Sperms count. Mill/ml	Total motility%	Diagnosis
48	9	50	OT
49	13	61	
50	7	55	
51	5	40	
52	13	46	
53	7	55	
54	24	30	AT
55	60	2	
56	23	17	
57	23	18	
58	17	2	
59	20	5	
60	33	21	
61	28	40	T
62	52	55	
63	37	66	
64	16	60	
65	38	60	
66	47	40	

The infertile group AT showed a poor sperm motility (asthenozoospermia) and abnormal sperm morphology (teratozoospermia) with normal sperm count. The infertile group T showed a defect in sperm shape (teratozoospermia) with normal sperm count and sperm motility. All infertility was of primary type except samples 8, 34 and 65 which were of the secondary type. In Table 3, the semen information of the control group is listed with normal sperm count (normozoospermia), shape and motility.

As shown in Table 4, thirteen substitutions were recorded in the ATP6 gene of all semen samples. All substitutions were transitions between purines or between pyrimidines with only one tranversion at the position C9060A. These substitutions were differentiated into 6 synonymous or silent and 7 non-synonymous (with changes in the translated amino acids in the translated ATP6 synthase). The synonymous substitutions were as follow: G8697A, G8856A, T8889C, T8952C, C9075T and C9060A. Among these mutations, G8856A was a novel as it was recorded in the infertile men only with a percentage of 1.5%. The two positions C9075T and C9060A were recorded in the healthy men only with a high percentage of 11.4%. The 7 non- synonymous substitutions were transitions. Cytosine was substituted with thymine at the position C8684T with a percentage of 4.5% in the infertile men only. At this site, threonine was changed to isoleucine at the amino acid position 53. At A8701G, substitution of threonine to alanine was recorded at the position 59 and it was recorded in both fertile and infertile men with an equal ratio (6.06%). The base substitution at the position G8860A resulted in a change of alanine 112 to threonine 112 in the infertile men only with a percentage of 1.5%. Leucine 84 was changed to phenylalanine at the position C8876T in the infertile men only with a percentage of 4.5%. At the position T8951C, valine 142 was substituted with alanine in both fertile (2.5%) and infertile people (1.5%). At the position G9055A, the infertile men only exhibited a novel substitution of alanine (aa = 177) to threonine with a percentage of 4.5%. Both fertile and infertile men showed a substitution of phenylalanine 193 with leucine 193 at the nucleotide position T9103C.

Table 5 shows the predicted haplogroups of the ATP6 gene. Nine haplogroups were detected (H2a, H1a, H5a, T, J2a, K1a, N1b, M25 and U7). The haplogroup H2a was overrepresented where 63.64% of either normal or infertile men belonged to this haplogroup. The haplogroups H5a, M25 and U7 were characteristic for infertile men who are suffering from oligoasthenoteratospermia where each of the three haplogroups appeared in those men with a percentage of 4.5% and did not appear in the normal men. The haplogroup K1a appeared in normal men only with a percentage of 4.5%. The other haplogroups appeared in both fertile and infertile men with higher representation in infertile men for N1b (4.5% infertile and 2.3% fertile) and T (3.03% infertile and 2.3% fertile) and lower representation for H1a (4.5% infertile and 6.8% fertile) and J2a (6.06% infertile and 9.09% fertile).

As shown in Table 6, two new non-synonymous mutations were recorded. The first was C8684T at which the amino acid changed from threonine to isoleucine. The second was C8776T at which the amino acid changed from leucine to phenylalanine. Table 7 indicates that there was no significant correlation between factors affecting fertility (smoking, exercise, diabetes, hypertension and varicocele) and ATP6 mutation.

Table 3: Samples of the fertile men (controls) with normal semen profile (normozoospermia) for sperm count and total motility percentage

Sample no	Sperms count. Mill/ml	Total motility%
67	120	60
68	46	60
69	48	62
70	52	66
71	120	60
72	105	58
73	100	80
74	47	72
75	25	80
76	52	66
77	26	85
78	25	70
79	60	80
80	60	80
81	54	70
82	38	80
83	40	62
84	33	45
85	30	73
86	45	65
87	30	80
88	40	70
89	20	75
90	47	70
91	46	58
92	64	72
93	50	80
94	85	62
95	120	60
96	46	60
97	48	62
98	50	55
99	120	60
100	105	58
101	100	80
102	47	72
103	120	60
104	46	60
105	48	62
106	52	66
107	50	55
108	20	55
109	5	50
110	28	60

Table 4: Percentage of SNP substitution (synonymous and non-synonymous) on ATP6 gene for fertile and diseased men as shown between brackets

	Total no = 110			Total no = 110	
Synonymous Substitution	Control = 44	infertile = 66	Non-synonymous substitution	Control = 44	infertile = 66
G8697A	1 (2.3%)	2 (3.03%)	C8684T (Thr^{53}Ile)	0	3 (4.5%)
G8856A	0	1 (1.5%)	A8701G (Thr^{59}Ala)	3 (6.8%)	4(6.06%)
T8889C	7 (15.9%)	3 (4.5%)	C8776T (Leu^{84}Phe)	0	3 (4.5%)
T8952C	5 (11.4%)	2 (3.03%)	G8860A (Ala^{112}Thr)	0	1 (1.5%)
C9075T	5 (11.4%)	0	T8951C (Val^{142}Ala)	1 (2.3%)	1 (1.5%)
C9060A	5 (11.4%)	0	G9055A (Ala^{177}Thr)	0	4 (6.06%)
			T9103C (Phe^{193}Leu)	4 (9.09%)	4(6.06%)

Table 5: The predicted haplogroups of the ATP6 gene for the infertile group. OAT) O = oligozoospermia, A= asthenozoospermia, T= Teratozoospermia and fertile control group

Predicted haplogroup	Case			Variants
	Control	Infertile	Disease	
H2a	63.64%	63.64%	T, OT, OAT, A	A8860G
H1a	6.80%	4.50%	O, AT	A8716C, T8736C, T8889C, T8952C, C9060A, C9075T, A8701G, A8860G
H5a	0	4.50%	OAT	A8860G, G9055A
T	2.30%	3.03%	OAT	G8697A, A8860G
J2a	9.09%	6.06%	T, AT, OAT	A8860G, T9103C, C9118T
K1a	4.50%	0	Normal	A8860G, T8952C, C9060A, C9075T
N1b	2.30%	4.50%	OAT	A8860G, A8836G

Table 6: Comparison of novel substitutions in ATP6 gene between this study and the previous studies

Ethnic group	Sample size	Number of detected substitutions	non-synonymous variations
Iran (Heidari *et al*., 2016)	fertile men (n = 159) infertile men (n = 72)	novel = 1	A9063G (synonymous)
New Zealand (Holyoake *et al*., 2001)	fertile men (n = 138) infertile men (n = 240)	novel = 15	T8821C, G8839A (non-synonymous), G8856A G8860A (non-synonymous), G9055A(non-synonymous)
Iraq (Mahdy and Abdul-Hassan, 2014)	asthenozoospermia (n = 56), Controls (n=10)	novel = 2	T8899C (synonymous) C8907T (synonymous)
Kumar and Sangeetha (2009)	Review article	Novel = 4	A8701G, G8860A, 8879, 9098
Saudi Arabia (this study)	Fertile men (n = 44) Infertile men (n = 66)	novel = 5	C8684T (non-synonymous), A8701G (non-synonymous) C8776T (non-synonymous) G8856A (synonymous) G8860A (non-synonymous) G9055A (non-synonymous)

Table 7: Correlation between some infertility factors and mutation in this study

Characteristics		Control (n = 44)	Patients (n = 66)	P[a]
Mean age (years; mean			1	
Smoking	Yes	24	25	0.5
	No	20	41	
Exercise	Yes	13	29	0.7
	No	31	37	
Diabetes	Yes	4	6	0.20
	No	40	60	
Hypertension	Yes	7	4	0.7
	No	37	62	
Varicocele	Yes	5	18	0.3
	No	39	48	
Mutation	Yes	15	23	-
	No	29	34	-

Discussion

From the forensic point of view, a biological sample collected from the crime scene could be a seminal fluid of an infertile man. This sample could acquire novel ATP6 gene SNPs and certain mtDNA haplogroups. These SNPs could be among the novel sites obtained herein and the haplogroups could be one of those recorded for the infertile people. Further investigations are, therefore, necessary to be conducted on more mtDNA data of infertile semen in order to assess the forensic contributions of male fertility. The present study was a preliminary or a pilot to construct a dozen of researches in the same direction either by tackling the mitogenome or by examining the STR profiles for the damaged sperms of the infertile males.

The specialized medical centre from which the samples were carefully collected conducted identification and classification of the pathological states of the collected samples. In spite of this approved identification, published evidence for classifying the samples into normospermic, teratospermic, oligospermic and asthenospermic as listed in WHO (2010) criteria was presented herein. Ji *et al*. (2017) and Liu *et al*. (2017) identified the different infertility cases according to sperm count and motility. Asthenospermia was identified by reduction in sperm motility lower than 40% motile sperms. Accordingly, Skowronek *et al*. (2017) identified oligozoospermia with less than 15 million

sperms per ml semen. These authors classified asthenozoospermia by total sperm motility less than 32%. They identified the morphological disorder of teratozoospermia by counting less than 4% normal sperms. Kumar and Sangeetha (2009) also classified the infertile sperms according to WHO (2010).

Comparing the present results with the previous studies, one can find a good evidence for applying the ATP6 gene in distinguishing between fertile and infertile men. However, certain debates between researchers were found in this perspective. The present study agreed with that of Holyoake *et al*. (2001) for samples from New Zealand in sharing the synonymous site G8856A and non-synonymous sites G8860A and G9055A. Our study agreed also with that of Kumar and Sangeetha (2009) in defining two sites in the infertile men which were the non- synonymous sites A8701G and G8860A. We did not find the SNPs identified by Heidari *et al*. (2016) and the SNPs identified by Mahdy and Abdul-Hassan (2014). The present study identified two novel non- synonymous SNPs in the Saudi men that were not identified before. These SNPs were C8684T and C8776T. In the first, the amino acid changed from thrionine to isoleucine at the amino acid position 53 while in the second, the amino acid changed from leucine to phenylalanine at the position 84. In agreement with the present study, a specific point mutation at the position 9055 is correlated with small semen quality parameters in 11% of studied cases (Holyoake *et al*., 1999; 2001; Shamsi *et al*., 2008). Kumar *et al*. (2009) supported the role of ATP6 gene in showing significant base changes in ATP gene of infertility cases compared to the controls. Contrary to the abovementioned evidence, Palanichamy and Zhang (2011) claimed that no correlation between the mtDNA substitutions and male infertility. These authors considered the published data are misleading application of the mitochondrial polymorphisms in male infertility diagnosis. Similarly, Pereira *et al*. (2007) analyzed the complete mtDNA of 20 asthenozoospermia patients and 23 whole mitogenomes of teratoasthenozoospermic males and confirmed no association between male infertility and mitogenomes.

To the best of our knowledge, few studies correlated ATP6 gene of infertile men with a specific mtDNA haplogroup. Those studies could support the application of mtDNA haplogroups of the infertile men in forensic caseworks. Mao *et al*. (2016) revealed that males with haplogroup Z might accumulate a higher risk of *in vitro* fertilization failure in Han Chinese men group. Similarly, the present study revealed that the OAT of Saudi men could be found in H5a, M25 and U7 ATP6 haplogroups. Inversely, some studies did not find a correlation between mtDNA mutations and specific haplogroups in men with reduced fertility (Pereira *et al*., 2005). However, those authors found a positive correlation between specific mtDNA SNPs in the hypervariable region of the d-loop and reduced male fertility. Since those authors sequenced only the hypervariable region, they were not able to correlate the male infertility with mtDNA haplogroup. Feng *et al*. (2013) found a strong correlation between the hypervariable d-loop region haplogroup R and sperm motility in Han population. Thus, some mtDNA fragments supported no correlation between mitogenome and male infertility and others supported this correlation. However, Bandelt (2008) revealed false correlation between mtDNA mutations, including the ATP6 gene and infertility. A recent study by Ji *et al*. (2018) found a unique non-synonymous substitution in ATP6 gene at the site C8684T to identify a non-obstructive azoospermia in 628 males. The defining haplogroup of this site was M8a. One of the M subhaplogroup (M25) was found in infertile men of the present study. Inversely, Skowronek *et al*. (2017) found that Americans with haplogroup F of the Y chromosome were highly frequent with teratospermia. The authors claimed that there was no correlation between sperm parameters and the haplogroups predicted from mitochondrial DNA.

Mitochondrial DNA supported significant insights into the maternal histories of human migrations (Jobling, 2012; Lippold *et al*., 2014). Few trials analyzed the possible correlation of mtDNA variants with male fertility (Skowronek *et al*., 2017). So far no forensic study has been conducted on the whole mtDNA of the infertile (low motile, morphological aberrations or low count) sperms. It could be possible that the infertile sperms show changes in the autosomal or Y-STR genotyping. Meanwhile, the mtDNA of the infertile sperm might acquire mutations with a forensic value. Refaat (2016) revealed that the Y-STR profiles of the azoospermic sperms were fully documented without any abnormalities. However, DNA fragmentation was characteristic for the infertile sperms and might be diagnostic for male infertility (Zeqiraj *et al*., 2018). There is no evidence that the DNA fragmentation in the infertile males could affect the STR profiling or the mtDNA. As the present study showed a correlation between the infertile sperms and specific ATP6 substitutions or mtDNA haplogroups, it could be possible that the obtained results exhibit forensic perspectives. Yukseloglu *et al*. (2018) used the mtDNA typing of the sperm cells collected individually from a sexual assault victim and the mtDNA of the perpetrator sperms taken from the victim epithelium by micromanipulation technique for human identification. These authors used the sequence of the d-loop region, which is well-documented as a forensic maker.

Conclusion

ATP6 gene could be used as evidence in distinguishing between fertile and infertile men since mtDNA of semen from infertile Saudi men, particularly those who were suffering from AOT, acquired novel ATP6 gene SNPs and specific haplogroups. However, some discrepancies in applying this gene in forensic caseworks were found. The present study agreed with some previous studies in finding an association between mtDNA mutations and specific haplogroups in men with reduced fertility and this is still a debate between researchers. The present study is, therefore, a clue for trialing intensive investigations on the mitogenome of damaged sperms in order to assess their forensic contributions.

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Author's Contributions

Safa A.M. Elsanousi: Conducted the practical part.

Hamad Sufyan and Murid Javed: Supported this study by providing samples, their consents and medical information.

Murid Javed: Revised the final draft.

Sayed A.M. Amer: Wrote the manuscript and followed up its publication.

Ethics

This article is original and contains unpublished material. The corresponding author confirms that all of the other authors have read and approved the manuscript and no ethical issues involved.

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