Correlation of Sperm Mitochondrial DNA 7345 bp and 7599 bp Deletions with Asthenozoospermia in Jordanian Population

Mazhar Salim Al Zoubi 1*, Ali M. Al-Talafha 2, Emad Al Sharu 3, Bahaa Al-Trad 2, Ayman Alzu'bi 1, Manal Issam AbuAlarjah 1, Qasem Shehab 4, Mohammad Alsmadi 5, Khalid M. Al-Batayneh 2*

1- Department of Basic Medical Sciences, Faculty of Medicine, Yarmouk University, Irbid, Jordan
2- Department of Biological Sciences, Faculty of Science, Yarmouk University, Irbid, Jordan
3- King Hussein Medical Centre, Royal Medical Services, Amman, Jordan
4- Department of Clinical Sciences, Faculty of Medicine, Yarmouk University, Irbid, Jordan
5- Department of Gynecology and Reproductive Medicine, Faculty of Medicine, Saarland University, Saarbrücken, Germany

Abstract

Background: Alterations in sperm mitochondrial DNA (mtDNA) affect the functions of some OXPHOS proteins which will affect sperm motility and may be associated with asthenozoospermia. The purpose of this study was to investigate the correlation between 7599-bp and 7345-bp sperm mtDNA deletions and asthenozoospermia in Jordan.

Methods: Semen specimens from 200 men including 121 infertile and 79 healthy individuals were collected at the Royal Jordanian Medical Services In-vitro fertilization (IVF) units. The mtDNA was extracted followed by mtDNA amplification. Polymerase chain reaction (PCR) was conducted for the target sequences, then DNA sequencing was performed for the PCR products. Chi-square, Fisher's and Spearman's tests were used to calculate the correlation.

Results: The results showed a significant correlation between the presence of 7599-bp mtDNA deletion and infertility where the frequency of the 7599-bp deletion was 63.6% in the infertile group compared to the fertile 34.2% (p<0.001, OR=3.37, 95% CI=1.860 to 6.108)). Additionally, the sperm motility showed a significant association with the frequency of the 7599-bp deletion (p=0.001, r=-0.887). The 7345-bp mtDNA deletion showed no association with the infertility (p=0.65, OR=0.837, 95% CI=0.464-1.51) or asthenozoospermia (p=0.98, r=0.008).

Conclusion: We demonstrated a significant correlation between asthenozoospermia and the 7599-bp mtDNA deletion but not the 7345-bp mtDNA deletion in the infertile men in Jordan. Screening for deletions in sperm mtDNA can be used as a pre-diagnostic molecular marker for male infertility.

Keywords: Asthenozoospermia, Gene deletion, Male infertility, Mitochondrial DNA, Sperm motility.

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Introduction

Infertility is the inability of a couple to conceive a child after one year of regular coitus without using contraception (1). It may be attributable to male or female factors (2). Approximately, 17% of couples are suffering from this condition (3). Among all infertility cases, 50% of cases are related to male factors (4).

The evaluation of the seminal fluid is generally based on the assessment of its volume, sperm count, liquefaction time, sperm motility, and mor-
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Sperm motility is essential in the fertilization process because it makes the sperm able to migrate from the vagina towards the fallopian tube and penetrate the ovum (6). Reduction in the motility of sperm is called asthenozoospermia which is a cause of male infertility that is characterized by decreased sperm motility below 40% (7). The rapid forward progressive movement of sperm is relying on energy (ATP) supplied by sperm mitochondria via the oxidative phosphorylation (OXPHOS) process (8). The OXPHOS genes are located on the mitochondrial DNA (mtDNA) genome. In humans, the mtDNA genome is a circular double-stranded DNA molecule of 16569 bp that encodes 13 polypeptides, two ribosomal ribonucleic acids (rRNAs) and 22 transfer ribonucleic acids (tRNAs) that play an essential role in the mitochondrial respiration and oxidative phosphorylation (9). Moreover, mtDNA has a large number of direct repeats of different sizes ranging between 4 to 17 bp (10) (Figure 1). Human mtDNA, compared to nuclear DNA, has different and unique properties like fast replication and lack of an efficient proofreading mechanism. Therefore, the rate of mtDNA alterations is expected to be 17 times higher than nuclear DNA (11, 12). Lack of DNA repair mechanism makes the mtDNA more vulnerable to the attack of normally formed free radicals and reactive oxygen species (ROS) (13).

The most common mtDNA deletion is the 4977 bp deletion, which tends to affect 40% of individuals with mitochondrial dysfunctions (14). Some studies in different populations reported a correlation between the 4977 bp mtDNA deletion and asthenozoospermia (12), whereas, in some other populations, there was no correlation between the 4977 bp deletion and the abnormalities in count, morphology or, motility of spermatozoa (15) (Figure 2). Moreover, other mtDNA deletions showed an association with poor sperm motility; for instance, 7599 and 7345 bp deletions have been studied by Kao et al. (9) and they reported a significant correlation between these mtDNA deletions and poor sperm motility. However, Talebi et al. (2018) reported that the 7599 bp mtDNA deletion was associated with asthenozoospermia, and no correlation was detected between the 7345 bp mtDNA deletion and reduced sperm motility (16).

The mtDNA 7599 bp deletion is located between 8642 and 16243 bp and is characterized by the presence of seven-nucleotide direct repeats (5’-CATCAAC-3’) at both sides whereas, 7345 bp mtDNA deletion lies between 9009 and 16354 bp (9). The molecular approaches have shown the association between 7599 and 7345 bp mtDNA deletion with functionality of some mitochondrial genes including ATPase8, ATPase 6, cytochrome c oxidase (COX) III, cytochrome b, NADH dehydrogenase (ND) 3, 4, 4L, 5, and 6. Also, those deletions include losing 8 tRNA genes (Figure 3) and all of these genes play a significant role in the

Figure 1. The human mitochondrial DNA (mtDNA). The genome consists of 37 genes coding for 13 polypeptides, 22 tRNAs, and 2 rRNAs (16S and 12S). The starting points of replication on the heavy strand (O+) and the light strand (O−) are indicated.

Figure 2. The 4977 bp (Common deletion) within the whole mtDNA. Deleted genes are indicated between the vertical red lines.

Figure 3. Locations and deleted genes within 7599 and 7345 bp mtDNA deletions. The sites of both deletions and the lost mtDNA genes appear; 7599 bp mtDNA deletion is between the two red lines, whereas 7345 bp mtDNA deletion is indicated within the blue lines.
Asthenozoospermia is considered the main cause of male infertility (17). Thus, screening of human mitochondrial DNA for mutations can be helpful in the molecular diagnosis of male infertility. Therefore, in this study, the association of 7599 bp human mitochondrial DNA deletions was investigated among Jordanian population.

Methods

Specimen collection and semen analysis: Semen specimens from 121 patients diagnosed with asthenozoospermia of ages between 18 and 40 years were collected between Jan 2017 and Dec 2017, and divided into six groups according to sperm motility; group one included 20 patients with 0% sperm motility (Completely immotile), group two included 20 patients with 1% to 5% sperm motility, group three involved 20 patients with 6% to 10% sperm motility, group four included 20 patients with 11% to 15% sperm motility, group five comprised 20 patients with 16% to 20% sperm motility, and finally, group six included 21 patients with 21% to 35% sperm motility.

Sample collection and all other protocols were approved by the local institutional review board at Yarmouk University. The consent form was signed by each participant after a verbal explanation of the objectives of the study.

Based on the exclusion criteria of the study, patients who drank alcohol and smoke cigarettes in addition to individuals who had a varicocele and aged more than 40 years were excluded from the study (10).

Semen specimens from 79 men with normozoospermia (High percentage of sperm motility, >50%) were collected which comprised the control infertile group. Similarly, samples were also subcategorized according to the percentage of sperm motility into three groups; group one included 25 samples with motility of 50% to 55%, group two involved 27 samples with sperm motility of 56% to 60%, and finally, group three included 27 samples with sperm motility of 61% to 75% (18).

Semen specimens were collected from in vitro fertilization (IVF) units of Jordanian Royal Medical Services at King Hussein Medical Center and Prince Rashid Ben Al-Hasan Military Hospital, where infertile patients provided their seminal fluid for evaluation. Seminal fluid specimens from all subjects were obtained by masturbation after three to five days of sexual abstinence. Semen specimens were collected in a special sterile, wide-mouth and non-toxic container and placed in the lab. Semen specimens were incubated at 37°C to allow liquefaction to occur for 30 min. Then, semen specimens were evaluated by a senior clinical embryologist following the guidelines of WHO laboratory manual for the examination and processing of human seminal fluid (19).

Semen specimens were fractionated using Percoll media (45% and 90% gradients) and centrifuged at 1000xg for 20 min. After that, the pellets were collected and washed two times with sperm washing medium to get rid of Percoll remnants, then spermatozoa were collected by centrifugation using microcentrifuge at 670xg for 10 min. Finally, the pellet containing spermatozoa was kept at −20°C until DNA extraction (20).

Sperm mtDNA amplification: Total DNA was extracted using QIAamp DNA Mini Kit (Qiagen, Germany) following the manufacturer’s instructions. After that, the mitochondrial DNA of spermatozoa was amplified using REPLI-g Mitochondrial DNA Kit (Qiagen, Germany) following the manufacturer’s recommendations. Then, the density ratio for amplified mitochondrial DNA from all specimens was measured, and specimens that had an optimal density ratio (260/280) close or equal to 1.8 were chosen and stored at −20°C.

Amplification of mtDNA: Polymerase chain reaction (PCR) was performed to confirm the occurrence of the mtDNA in the extracted genome reaction; for this purpose, the sample was prepared with a total volume of 30 µl containing 15 µl of 2X master mix (GoTaq, Promega, USA), 1 µl of each primer, 3 µl of diluted amplified mtDNA and 10 µl of nuclease-free water. The PCR was performed using specific primer pair (F1-R1) (Table 1), and the fragment size of the amplicon was expected to be 414 bp (Table 1). PCR was carried out using a thermal cycler (Bioer, China) upon the following program; the initial denaturation step was done at 95 °C for 2 min followed by 40 cycles of denaturation at 95 °C for 30 s, annealing step at 55 °C for 30 s, and extension at 72 °C for 1 min. Finally, the reaction was ended with the final extension step at 72 °C for 5 min.

The resulted PCR products were resolved by electrophoresis with 1% agarose gel at 100 V for 45 min using 1X Tris-Borate-EDTA (TBE) buffer. Next, they were visualized by UV and a 50-bp DNA marker was applied.
Detection of 7599 and 7345 bp mtDNA deletions: To detect the existence of 7599 and 7345 bp deletions in mtDNA samples, PCR reaction was performed using F4-R3, F2-R3, and F5-R4 primers pairs, respectively (Table 1), and the length of the PCR products was predicted to be 406, 756 bp, and 555 bp, respectively (Table 1). PCR amplification to detect both deletions was done and each sample in a total volume of 30 µl contained 15 µl of 2X master mix (GoTaq, Promega, USA), 1 µl of each one of the specific primers, 3 µl of diluted amplified mtDNA and 10 µl of nuclease-free water. PCR conditions for detection of 7599 bp deletion were started with initial denaturation step at 95 °C for 2 min followed by 35 cycles of denaturation at 95 °C for 30 s, annealing at 55 °C for 30 s and extension at 72 °C for 1 min. Then, the final extension step was performed at 72 °C for 5 min.

Next, PCR conditions to detect 7345-bp deletion were started with an initial denaturation step at 95 °C for 2 min followed by 40 cycles of denaturation at 95 °C for 30 s, annealing at 60 °C for 30 s followed by an extension step at 72 °C for 1 min. Finally, a final extension step was performed at 72 °C for 5 min.

The resulted PCR products were resolved by electrophoresis with 1% agarose gel at 100 V for 45 min using 1X Tris-Borate-EDTA (TBE) buffer. Next, they were visualized by UV and DNA marker of 50 bp was applied.

Table 1. Oligonucleotide primers used for mtDNA confirmation and PCR amplification of 7599 and 7345 bp deletions (Kao et al., 1998)

| Primers | Primer sequences | Primer pairs | Amplified position | Amplicon size (bp) |
|---------|-----------------|--------------|--------------------|-------------------|
| F1      | 5'-AACATACCCATGCGCAACT-3' | (F1-R1) mtDNA | 3304–3717         | 414               |
| R1      | 5'-GGCTACCTGTCGATCGTGGC-3' |             |                    |                   |
| F5      | 5'-ACGAAAATCTGTTGGCCTCA-3' | (F5-R4) 7345 bp | 8531–16, 430      | 555               |
| R4      | 5'-TGCGGAGATATTGATTTTACGC-3' |             |                    |                   |
| F2      | 5'-TGAAACCTACGAGTACACGGA-3' | (F2-R3) 7599 bp | 7901–16, 255      | 756               |
| R3      | 5'-CTTTGGAGTTCGTGATTTGATG-3' |             |                    |                   |
| F4      | 5'-GCCCGTATTTACCCTATAGC-3' | (F4-R3) 7599 bp | 8251–16, 255      | 406               |
| R3      | 5'-CTTTGGAGTTCGTGATTTGATG-3' |             |                    |                   |

Statistical analysis: SPSS software was used for data analysis. Frequency was calculated for 7599 and 7345 bp mtDNA deletions among the study population. Spearman’s correlation analysis was conducted to evaluate the relationship between the presence of the corresponding deletions and the reduction in sperm motility. Furthermore, Fisher’s exact test was applied to calculate the odds ratio with 95% confidence intervals. The p-value was considered significant if it was ≤0.05. Graphs were drawn using GraphPad Prism 7.00 software.

Results

The study population consisted of 200 individuals including 121 infertile and 79 fertile men that were classified according to the sperm motility percentage into subgroups as shown in tables 2 and 3. There was no significant difference in the mean age between the infertile and fertile groups (p>0.05). The mean age of the infertile group was 34.4 years, whereas, the mean age for the control group was 32.7. The PCR amplification of the target sequences was confirmed by detection of the target bands on 2% agarose gel as shown in figure 4.

The results showed a significant difference in the presence of the 7599 bp mtDNA deletion in 77 infertile individuals (63.6%) compared to only 27 individuals (34.2%) of the control group, calculated by Fisher’s exact test (p<0.001) (OR=3.37, 95% CI=1.860 to 6.108) (Table 2). However,
There was no significant difference in the presence of the 7345 bp mtDNA deletion in 41 infertile individuals (33.9%) compared to 30 fertile individuals (38.0%) of the control group (p=0.650) (OR=0.837, 95% CI=0.464-1.51) (Table 2).

Also, infertile and fertile individuals were sub-categorized into nine groups based on the sperm motility percentage as shown in Tables 3 and 4. Using Spearman’s correlation, the frequency of 7599 bp mtDNA deletion in the categorized groups showed a significant correlation between the sperm motility percentage and the presence of the 7599 bp mtDNA deletion (p=0.001, r=-0.887) (Table 3). However, the frequency of 7345 bp mtDNA deletion in the categorized groups showed no significant correlation between sperm motility percentage and the presence of the 7345 bp mtDNA deletion (p=0.98, r=0.008) (Table 4). Direct DNA sequencing was performed to verify the deletion sites in the mtDNA as shown in figures 5 and 6.

Collectively, the current cohort showed that the prevalence of 7599 bp mtDNA deletion was notably higher and significantly associated with asthenozoospermia-induced infertility.

**Discussion**

Asthenozoospermia is considered a major cause of male infertility worldwide that is attributed to

### Table 3. Frequencies of 7599 bp mtDNA deletion in infertile and fertile group according to sperm motility

| Motility | Number of cases | Presence of deletion | Percentage | p-value (spearman’s correlation) |
|----------|-----------------|----------------------|------------|--------------------------------|
| 0%       | 20              | 14                   | 70%        | 0.001 (r=-0.887)               |
| 1-5%     | 20              | 15                   | 75%        |                                |
| 6-10%    | 20              | 13                   | 65%        |                                |
| 11-15%   | 20              | 11                   | 55%        |                                |
| 16-20%   | 20              | 14                   | 70%        |                                |
| 21-35%   | 21              | 10                   | 48%        |                                |
| 50-55%   | 25              | 13                   | 52%        |                                |
| 56-60%   | 27              | 10                   | 37%        |                                |
| 61-75%   | 27              | 4                    | 15%        |                                |
| Total    | 200             | 104                  |            |                                |

### Table 4. Frequencies of 7345 bp mtDNA deletion in infertile and fertile group according to sperm motility

| Motility | Number of cases | Presence of deletion | Percentage | p-value (spearman’s correlation) |
|----------|-----------------|----------------------|------------|--------------------------------|
| 0%       | 20              | 9                    | 45%        | 0.98 (r=0.008)                  |
| 1-5%     | 20              | 7                    | 35%        |                                |
| 6-10%    | 20              | 4                    | 20%        |                                |
| 11-15%   | 20              | 4                    | 20%        |                                |
| 16-20%   | 20              | 13                   | 65%        |                                |
| 21-35%   | 21              | 4                    | 19%        |                                |
| 50-55%   | 25              | 12                   | 48%        |                                |
| 56-60%   | 27              | 12                   | 44.4%      |                                |
| 61-75%   | 27              | 6                    | 22.2%      |                                |
| Total    | 200             | 71                   |            |                                |
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the insufficient provision of energy (ATP) to spermatozoa through the OXPHOS mechanism of the mitochondria located in the middle piece of the spermatozoon (7, 21). The mtDNA is responsible for encoding four of the five major complexes of OXPHOS complexes (22). Considering the presence of a single copy of the mtDNA in each mitochondrion (23), naturally, the mtDNA lacks efficient proofreading and repairing mechanisms, so the rate of mutation is much higher than the nuclear DNA (13). Simultaneously, the mtDNA is susceptible to be attacked by ROS which is generated from the normal mitochondrial respiration process, and as a consequence, the mtDNA may be damaged due to elevated oxidative stress which affects sperm functions mainly the sperm motility (24). Several mtDNA deletions have been reported to be highly correlated with male infertility in patients with asthenozoospermia, such as the 4977 bp, 7599 bp, and 7345 bp deletions. In a previous study, a significant association between the mtDNA 4977 bp deletion and the asthenozoospermia in Jordanian population was reported (25).

Additionally, a highly significant association was found between the presence of 7599 bp mtDNA deletion and the reduction in sperm motility in the same study. However, the 7345 bp mtDNA deletion did not show any significant correlation with men's infertility and asthenozoospermia. In addition, our DNA sequencing results confirmed the loss of essential genes that are responsible for the OXPHOS process. The current results are consistent with a recently published study in Iran (16). The researchers found a significant association between the incidence of 7599 bp mtDNA deletion and asthenozoospermia and also 7599 bp mtDNA deletion was found in the individuals with oligoasthenoteratozoospermia; the occurrence of this deletion is associated with the loss of the ATPase 6 gene in addition to the other OXPHOS genes of the mtDNA, which consequently affect the sperm motility. In addition, they did not find any significant association between the prevalence of 7345 bp deletion and reduction in sperm motility (16). These findings were supported by other researchers who found that the existence of large-scale mtDNA deletions such as 7599 bp and 7491 bp was markedly associated with reduced sperm motility because they are responsible for the loss of some mitochondrial genes affecting male fertility. They also proposed that screening mtDNA for such deletion may provide clear evidence behind the etiologies of male infertility (26). Moreover, the role of the 7599 and 7345 bp mtDNA deletions in male infertility was investigated by Kao et al. (9). They found a significant association between the incidence of 7599 bp deletion in the sperm mtDNA and the reduction in sperm motility which is supported by our findings (9). Kao et al. reported a significant association between the incidence of 7345 bp deletion and asthenozoospermia (9) which is inconsistent with our results. Compared to our findings, the discrepancy in the results may be due to small sample size which consisted of 36 patients compared to 121 that were included in the current study.
However, they reported that these deletions are responsible for the loss of some genes in OXPHOS process. Such a loss will affect the activity of the mitochondria in energy production and therefore impairs sperm motility which as a consequence elevates the proportion of male infertility (9).

Moreover, the results of our study are inconsistent with the results of a research study conducted by John et al. (15). The existence of 7491 bp mtDNA deletion in spermatozoa was investigated in 33 semen samples. They proposed that using magnetic beads coated with a special antibody for the separation of sperm may help in the elimination of leukocytes which are considered as contaminants of sperm specimens. As a result, they suggested that there was no association between these mtDNA deletions and sperm count, morphology, or motility (15).

According to the current findings, the difference in the incidence of 7345 bp mtDNA deletion among the patients’ group rather than control subjects does not necessarily mean that this deletion clinically contributes to sperm motility; in fact, partial correlation analysis revealed that 7599 bp mtDNA deletion is significantly correlated with motility, while 7345-bp mtDNA deletion is not significantly correlated with motility among study subjects. In support of that, the 7599 bp deletion is even more significantly correlated with motility when compared to 7345 bp deletion.

The contradiction in the results of various studies can be attributed to variations in the methodology, especially the sperm separation protocols. Moreover, the prevalence of the deletions in sperm mtDNA among control groups may be due to the effect of the environmental factors such as elevated scrotal temperature, the exposure to pesticides, radiation and cigarette smoking which lead to accumulation of the oxidative stress and therefore an increase in the chance of DNA breaks and damage. Also, some medications and antibiotics can affect male fertility. Furthermore, the sample size in the mentioned studies was small and the variation between populations may be a factor behind the contradictory results.

Therefore, the reported mtDNA deletions have to be studied on a larger scale population with consistent techniques of separation and processing to emphasize the role of these mutations in the development of male infertility. From the molecular point of view, the reported mutations are associated with the deletion of a large portion of mtDNA responsible for the expression of OXPHOS complexes. In light of that, it is crucial to clarify the presence of these large scale mutations in the control groups. Generally, the presence of these mutations in the control (Fertile) males can be attributed to the percentage of these mutations in the spermatozoa. That is supported by the correlation between the incidences of the mtDNA deletions and sperm motility.

**Conclusion**

A high prevalence of mtDNA deletion was reported in infertile Jordanian males in this study and a highly significant association was found between the presence of 7599 bp mtDNA deletion and asthenozoospermia. However, no significant correlation was reported between the presence of 7345 bp mtDNA deletion and asthenozoospermia. Our current findings support the previous report of the association between the mtDNA deletion and the occurrence of asthenozoospermia in Jordanian population. Further investigations are required to study other mtDNA mutations for their association with male infertility.

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**Conflict of Interest**

We declare there is no conflict of interest.

**References**

1. Brugo-Olmedo S, Chilik C, Kopelman S. Definition and causes of infertility. Reprod Biomed Online. 2001;2(1):41-53.
2. Demain LA, Conway GS, Newman WG. Genetics of mitochondrial dysfunction and infertility. Clin Genet. 2017;91(2):199-207.
3. Hull MG, Glazener CM, Kelly NJ, Conway DI, Foster PA, Hinton RA. et al. Population study of causes, treatment, and outcome of infertility. Br Med J (Clin Res Ed). 1985;291(6510):1693-7.
4. Kumar N, Singh AK. Trends of male factor infertility, an important cause of infertility: a review of literature. J Hum Reprod Sci. 2015;8(4):191-6.
5. Wang C, Swerdloff RS. Limitations of semen analysis as a test of male fertility and anticipated needs from newer tests. Fertil Steril. 2014;102(6):1502-7.
6. Ortega C, Verheyen G, Raick D, Camus M, Devroye P, Tournaye H. Absolute asthenozoospermia and
ICS: what are the options? Hum Reprod Update. 2011;17(5):684-92.

7. Baker HW, Clarke GN. Sperm morphology: consistency of assessment of the same sperm by different observers. Clin Reprod Fertil. 1987;5(1-2):37-43.

8. Amaral A, Lourenco B, Marques M, Ramalho-Santos J. Mitochondria functionality and sperm quality. Reproduction. 2013;146(5):R163-74.

9. Kao SH, Chao HT, Wei YH. Multiple deletions of mitochondrial DNA are associated with the decline of motility and fertility of human spermatozoa. Mol Hum Reprod. 1998;4(7):657-66.

10. Ambulkar PS, Waghmare JE, Chaudhari AR, Wankhede VR, Tarnekar AM, Shende MR, et al. Large scale 7436-bp deletions in human sperm mitochondrial DNA with spermatozoa dysfunction and male infertility. J Clin Diagn Res. 2016;10(11):GC09-GC12.

11. Pandey R, Mehrrota D, Catapano C, Choubey V, Sarin R, Mahdi AA, et al. Association of mitochondrial deoxyribonucleic acid mutation with polymorphism in CYP2E1 gene in oral carcinogenesis. J Oral Biol Craniofacial Res. 2012;2(1):4-9.

12. Kao S, Chao HT, Wei YH. Mitochondrial deoxyribonucleic acid 4977-bp deletion is associated with diminished fertility and motility of human sperm. Biol Reprod. 1995;52(4):729-36.

13. Aitken RJ, Clarkson JS. Significance of reactive oxygen species and antioxidants in defining the efficacy of sperm preparation techniques. J Androl. 1988;9(6):367-76.

14. Shamsi MB, Kumar R, Bhatt A, Bamezai RN, Kumar R, Gupta NP, et al. Mitochondrial DNA mutations in etiopathogenesis of male infertility. Indian J Urol. 2008;24(2):150-4.

15. St John JC, Jokhi RP, Barratt CL. Men with oligoasthenoteratozoospermia harbour higher numbers of multiple mitochondrial DNA deletions in their spermatozoa, but individual deletions are not indicative of overall aetiology. Mol Hum Reprod. 2001;7(1):103-11.

16. Talebi E, Karimian M, Nikzad H. Association of sperm mitochondrial DNA deletions with male infertility in an Iranian population. Mitochondrial DNA A DNA Mapp Seq Anal. 2018;29(4):615-23.

17. Marmor D, Grob-Menendez F. Male infertility due to asthenozoospermia and flagellar anomaly: detection in routine semen analysis. Int J Androl. 1991;14(2):108-16.

18. Bahrehmand Namaghi I, Vaziri H. Sperm mitochondrial DNA deletion in Iranian infertiles with asthenozoospermia. Andrologia. 2017;49(3).

19. World Health Organisation. WHO laboratory manual for the examination and processing of human semen. 5th ed. Geneva: World Health Organisation; 2010. 286 p.

20. Jeremias P, Rodakis GC. Correlation of the 4977 bp mitochondrial DNA deletion with human sperm dysfunction. BMC Res Notes. 2009;2:18.

21. Gibbons I. Sperm motility: mechanisms and control. In: Andre J, editor. The Sperm Cell. Berlin: Springer; 1983. p. 304-14.

22. Bornstein B, Huertas R, Ochoa P, Campos Y, Guillen F, Garess R, et al. Mitochondrial gene expression and respiratory enzyme activities in cardiac diseases. Biochim Biophys Acta. 1998;1406(1):85-90.

23. Hecht NB, Liem H, Kleene KC, Distel RJ, Ho Sm. Maternal inheritance of the mouse mitochondrial genome is not mediated by a loss or gross alteration of the paternal mitochondrial DNA or by methylation of the oocyte mitochondrial DNA. Dev Biol. 1984;102(2):452-61.

24. Aitken RJ. Human spermatozoa: revelations on the road to conception. F1000prime Rep. 2013;5:39.

25. Al Zoubi MS, Al-Batayneh K, Alsmadi M, Rashed M, Al-Trad B, Al Khateeb W, et al. 4,977-bp human mitochondrial DNA deletion is associated with asthenozoospermic infertility in Jordan. Andrologia. 2020;52(1):e13379.

26. Hosseinzadeh Colagar A, Karimi F. Large scale deletions of the mitochondrial DNA in astheno, asthenoterato and oligoasthenoterato-spermic men. Mitochondrial DNA. 2014;25(4):321-8.