

Comparison between *SPATA18* and *P53* Gene Expressions in The Sperm Cells Obtained from Normospermic and Asthenospermic Samples: A Case-Control Study

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Abstract

Background: Improving sperm motility results in increasing the success of a treatment cycle. Recently, sperm RNA has been used for diagnostic purposes such as whole seminal fluid, sperm analysis, and sperm quality test in patients undergoing *in vitro* fertilization/intracytoplasmic sperm injection (IVF/ICSI). *SPATA18*-*P53* pathway is considered an essential pathway related to sperm mitochondria, which controls mitochondrial quality by eliminating its oxidative proteins. Oxidative stress may decrease sperm motility and affect sperm quality negatively due to an increase in *P53* expression. *SPATA18* protein is found in satellite fibers related to outer dense fibers in the middle piece of sperm. The downregulation of *SPATA18* in the asthenospermia group can represent this gene's critical function in sperm motility and fertility. The present study aimed to assess the relationship between *SPATA18* and *P53* gene expression in sperm cells obtained from normospermia and asthenospermia.

Materials and Methods: In this case-control study, the quantitative real-time polymerase chain reaction (RT-PCR) technique was used to measure the *SPATA18* and *P53* gene expression level in sperm samples collected from 21 patients and 63 healthy individuals. Further, the sperm DNA fragmentation assay (SDFA) kit was applied to determine the relative apoptosis level in cells and evaluate the biochemical information related to the patients' sperm samples. Furthermore, all the participants completed the consent form, and the ethics committee confirmed the study.

Results: Based on the results, the *P53* and *SPATA18* gene expression levels in most of the samples, in which motility was less than 40%, increased and decreased ($P \leq 0.001$), respectively.

Conclusion: The *SPATA18* and *P53* gene expression levels increased and decreased in the asthenospermic patients, respectively, compared to the control group. Thus, the *P53* and *SPATA18* expression levels can be used as an appropriate marker for diagnosing sperm motility in males.

Keywords: Apoptosis, Asthenosperm, Normosperm, *P53*, *SPATA18*

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Introduction

Recently, sperm RNA has been applied for diagnostic purposes such as whole seminal fluid, sperm analysis, and sperm quality test in patients undergoing *in vitro* fertilization/intracytoplasmic sperm injection (IVF/ICSI). Sperm RNA is considered a potential marker for diagnosing sperm abnormalities and fertility capability in infertility clinics (1). Based on the latest information provided by the World Health Organization, normal sperm parameters are considered 15 million sperms with 4% normal morphology and 40% motile sperm or at least 32% with progressive motility per ml of seminal fluid (2). The infertility distribution caused by male factors varies between 20-70%, and the percentage of infertile males ranges between 2.5-12% (3-9).

Asthenospermia is a common reason for male infertility, which is diagnosed by reduced sperm motility

in new ejaculation (8). The maximum frequency of genetic factors recognized in male infertility (25%) is observed in asthenospermia (10). Three main factors, including decreased sperm count, motility power, and abnormal sperm morphology, are raised in male infertility. Selecting normal and mature sperms is considered useful in assisted reproduction techniques (11-13).

Mieap is considered another name for *SPATA18*. The genomic position for *SPATA18* gene is on the chromosome 4 (GRCh38/hg38), with the 46,002 bp linear DNA length and the 14 exons. The genomic position for *P53* gene is on the chromosome 17 (GRCh38.p13), with the length of 19149 bp linear DNA and 12 exons. It is assumed that *SPATA18* is a *P53* inducible protein, the transcription of which is directly regulated by the *P53* tumor suppressor. Mieap controls mitochondrial

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quality by repairing or removing unhealthy mitochondria through the Mieap-induced accumulation of lysosome-like organelles within mitochondria (MALM) or Mieap-induced vacuole (MLV), respectively (14, 15). Inactivating *P53* or *Mieap* disturbs MALM and MLV severely, leading to the accumulation of unhealthy mitochondria. Mitochondria are necessary for intracellular signaling and cellular energy supply after stress (16, 17).

Crosstalk is available between the nucleus and mitochondria during stress events (16). Reactive oxygen species (ROS) are produced as side-products during the oxidative phosphorylation process, the overproduction of which can play a role in mitochondrial damage and stress (16, 18). Mitophagy is considered an effective mechanism for controlling mitochondrial quality since it can optionally remove unwanted or damaged mitochondria (16, 19). Mitophagy plays a role in basal mitochondrial turnover and eliminates damaged mitochondria under stress (16, 20).

Additionally, these organelles are regarded a major source of intracellular ROS, including highly reactive free oxygen radicals like hydroxyl radical (OH•) and superoxide anion (O₂•⁻), as well as stable non-radical oxidants such as hydrogen peroxide (H₂O₂) (21). ROS is commonly created as the by-products of oxidative phosphorylation (21, 22). The generation of excessive ROS in mitochondria (mtROS) causes oxidative damage to lipids, proteins, and DNA and may lead to apoptosis (21, 23). Further, ROS accumulation can cause various diseases like degenerative disorders and cancer. Based on recent reports, elevated levels of mtROS can increase cancer cell invasion and metastasis through activating different major signaling pathways and transcription factors (21). Increasing oxidative stress, such as oxygen free radicals and ROS, can negatively affect sperm quality (24). Mitochondria include cardiolipin (CL), which is considered an organelle-specific phospholipid that carries 40 fatty acids with a strong preference for unsaturated chains (25-27). Further, TPCL is associated with acrosome, a sperm-specific organelle, during spermiogenesis, along with a subset of authentic mitochondrial proteins such as Suox, Ant4, and *SPATA18* (27).

Proteins such as caspase -1, 3, 7, 8, and 9 and aquaporin-7 are involved in regulating mitochondrial function in the apoptotic pathway and decreasing the sperm volume. Apoptosis may be associated with decreasing sperm motility (28). *SPATA18* protein is found in satellite fibers related to outer dense fibers in the middle piece of sperm. Decreasing *SPATA18* expression in the asthenospermia group can represent this gene's critical function in sperm motility and fertility (29, 30).

Based on Dan et al. (16), *SPATA18* expression is an essential player in the mitophagy process after DNA damage.

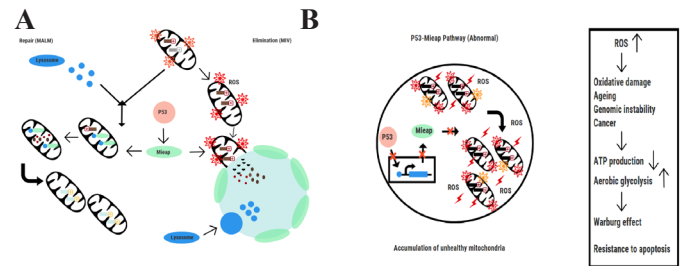


Fig.1: The Cross talk between *P53* and *Mieap* in mitochondria. **A.** The hypothetical model of *P53*-*Mieap* pathway for mitochondrial quality by which *Mieap* controls mitochondrial quality by repairing or removing unhealthy mitochondria through generating MALM or MIV, respectively (14, 15), and inactivating *P53* or *Mieap* disturbs MALM, and MLV severely leads to the accumulation of unhealthy mitochondria and **B.** Hypothetical model for inactivating *P53*-*Mieap* pathway by which ROS surfaces increase under severe oxidative stress such as oxygen free radicals, leading to DNA damage and apoptosis induction, and finally the disruption in the function of *P53*-*Mieap* (14). MALM; *Mieap*-induced accumulation of lysosome-like organelles within mitochondria, MIV; *Mieap*-induced vacuole, and ROS; Reactive oxygen species.

Considering the changes in *SPATA18* gene expression in normospermic and asthenospermic cells and the effect of the gene expression on *P53*-induced apoptosis, the present study aimed to assess the possibility of gene expression and its effect in generating apoptosis in sperm cells.

Materials and Methods

Sampling, classification, and characterization of samples

In this case-control study, the count and motility of the sperm samples of the 84 participants, who were referred to Bu Ali Laboratory in Zanjan for 6 months from June-November of 2020, were written on the day of sample preparation and the data were sorted from minimum motility to maximum one. From the total 84 samples were assessed in the present study, of which 21 and 63 were related to the patients and healthy individuals, respectively. Also, to appropriately compare gene expression levels between the asthenospermia and normospermia samples, the normospermic samples were divided into three subgroups, each of them including 21 samples based on their motility range, including [41-55] subgroup A, [55-69] subgroup B, and [69-83] subgroup C. Since the population included 21 asthenospermic samples and 63 normospermic ones, the control group with 63 members was divided into three subgroups to compare the 21-member asthenospermia group with each of the control subgroups statistically.

Primer design

Table 1 presents the sequence of primers using Oligo 7 software. In this table, the content of each reaction and the time and temperature of each cycle is shown.

In Table 2, the *P53* and *SPATA18* gene expression levels are compared between the asthenospermia group with the asthenospermic samples and the three normospermia subgroups. Table 3 shows the sperm DNA fragmentation assay (SDFA) results concerning gene expression in two groups of fair to low and good fertility potential.

The sperm samples were analyzed using HFTCASA Computer Aided Semen Analysis System software, 8.00 (31). A t test was implemented to compare the results using SPSS 22 (IBM Company, USA). Which was evaluated using the Kolmogorov-Smirnov test. To compare two groups from t test and to compare 3 groups from one-way analysis of variance. The abbreviation ANOVA is used. The significance level was considered 0.05, and the SDFA results were calculated using REST 2009 software. In this software, REST RG mode is used for data analysis.

Total RNA was extracted using EZ-10 Spin Column Total RNA Mini-Preps Kit (BioBasic Inc., USA) based on the Sperm RNA Company's guidelines.

cDNA synthesis

The cDNA complementary strand was created using the RNA extracted by the Takara kit based on the company's guidelines. Additionally, cDNA was synthesized immediately after extracting RNA based on the Takara Company's kit protocol (cat.no RR037Q).

Real-time polymerase chain reaction

The Rotor-Gene (Q) real-time PCR machine (QIAGEN) was used in the present study.

Steps of real-time polymerase chain reaction

Real-time PCR was conducted for the target and control

genes in two separate, paired tubes. The mixture volume is provided in Table 1.

Halosperm® G2 kit (HT-HSG2, halotech)

Of the 84 samples, 24 were randomly assessed using the SDFA kit. Additionally, the Halosperm® G2 kit (HT-HSG2, Halotech) was used based on the company's guidelines for sperm DNA fragmentation assay (SDFA).

Reference range of SDFA

- Samples with SDF<15%: These samples were assayed to have a deficient fragmentation level and ranked good.
- Samples with 15%<SDF<30%: These samples were assayed to have a low fragmentation level and ranked medium.
- Samples with 30%<SDF: These samples were assayed to have a high fragmentation level and ranked abnormal.

Results

A significant difference was observed regarding the *P53* and *SPATA18* gene expression levels between the asthenospermia group and the subgroups A, B, and C of normospermic samples. As shown in Table 2A, by decreasing motility rates in the asthenospermic samples led to lower *SPATA18* and higher *P53* gene expression levels compared to the normospermic samples.

Table 1: The sequence of primers and the condition of optimized Real-Time PCR reaction

Primer	Primer length (5'-3')	Length of created piece (bp)
<i>GAPDH</i>	F: GGTCATCATCTCTGCCCCCT R: AGGCAGGGATGATGTTCTGG	276
<i>SPATA18</i>	F: GTTCAGCGATTCCATATCCAGGC R: TCGACCCACATAAGATGGTGTC	192
<i>P53</i>	F: ATAGTGTGGTGGTGCCCTATGAGC R: TTCCAGTGTGATGATGGTGAGGAT	134

Component	Vol./reaction (µl)	Final concentration (µM)
2X Master Mix RealQ Plus	10	1x
Forward primer	0.5 (0.25-2.5)	0.1 (0.05-0.5)
Reverse primer	0.5 (0.25-2.5)	0.1 (0.05-0.5)
PCR-grade H ₂ O	7	-
Template cDNA	2	0.1
Total	20	-

Cycles	Duration of cycle	Temperature (°C)
1 for activation TEMPase	15 minutes	95
40	15 seconds	95
	30 seconds	52
	30 seconds	72

Table 2: The obtained P value of *P53* and *SPATA18* gene expression levels in the studied groups to determining statistical significance. The comparison of the *P53* and *SPATA18* gene expression levels between the asthenospermia and three normospermic subgroups samples, and the simultaneous assessment of significance of expression *P53*, *SPATA18* genes in all the three subgroups (a, b, c) and total samples (d)

	Gene	P value		Gene	P value	
		<i>P53</i>			<i>SPATA18</i>	
a. The mean difference of the asthenospermia and normospermia subgroup A	12.86 _{ΔCT}	0.023		-14.06 _{ΔCT}	0.001	
b. The mean difference of the asthenospermia and normospermia subgroup B	36.72 _{ΔCT}	0.025		-14.10 _{ΔCT}	0.001	
c. The mean difference of the asthenospermia and normospermia subgroup C	33.90 _{ΔCT}	0.004		-14.05 _{ΔCT}	0.001	
d. The mean difference of the asthenospermia and normospermia total subgroups	12.86 _{ΔCT}	0.023		-14.07 _{ΔCT}	0.00000*	

* P value decreases to zero to five decimal places

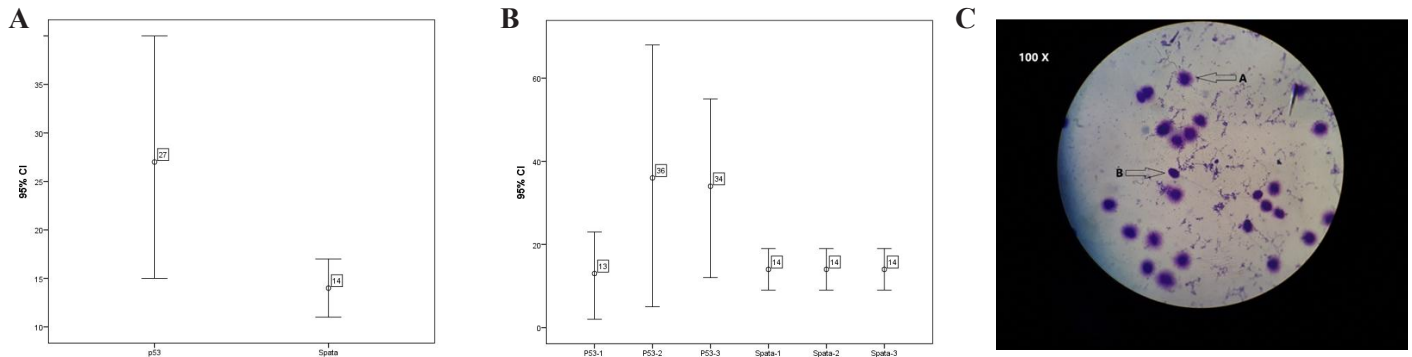


Fig.2: The results of the comparison of *P53* and *SPATA18* gene expressions obtained by both RT-PCR and S DFA methods. **A.** The mean difference of the asthenospermia and normospermia groups regarding *P53* and *SPATA18* genes in all the three subgroups. **B.** The mean difference of the asthenospermia and normospermia groups in *P53* and *SPATA18* genes in each of the three subgroups. **C.** Sperms (A) with halo (healthy and without DNA fragmentation) and (B) without halo (with DNA fragmentation) with ×100 magnification. CI; Confidence Interval of mean, S DFA; Sperm DNA fragmentation assay, and RT-PCR; Real-time polymerase chain reaction.

Table 3: The S DFA results concerning gene expression in two groups of fair to low and good fertility potential by using REST 2009 software

Gene	Reaction efficiency	Expression	P value
<i>GAPDH</i>	0.7124	1.000	
<i>P53</i>	0.6548	1.617	0.748
<i>SPATA18</i>	0.6014	7.012	0.078

The *P53* and *SPATA18* gene expression levels were compared between the asthenospermia group and the three subgroups of normospermic samples. Based on the real-time PCR technique results, the *P53* gene expression level increased ($P \leq 0.01$ up, especially Table 2 subgroup C) whereas the *SPATA18* expression level decreased ($P \leq 0.01$ down, Table 2 all subgroups). Furthermore, based on the analysis of expression level of total normospermic with asthenospermic samples, the $P \leq 0.05$ and $P < 0.001$ were obtained for *P53* and *SPATA18*, respectively, and the difference was significant due to the high sample size (Table 2). However, no significant difference was observed concerning gene expression between the good and fair to low fertility potential groups (Table 2). In the healthy groups, the possibility of asthenospermia in males increased by decreasing their motility range to 40. Comparing the data related to the three groups demonstrated that the *P53* and *SPATA18* expression levels in patients with motility between 12.2 and 30 increased and decreased, respectively.

Thus, the *SPATA18* gene expression level hypothesis was accepted in normospermic and asthenospermic cells by

considering the effect of its expression in generating *P53*-induced apoptosis and assessing the *SPATA18* and *P53* gene expression levels, which was different in sperm cells.

Based on the results, a significant difference was observed between the asthenospermia and normospermia groups. Figures 2A and 2B display the mean difference of the asthenospermia and normospermia groups regarding *P53* and *SPATA18* genes in all and each of the three subgroups, respectively.

Discussion

In the recent decade, the recognition of male reproductive function and the effect of malefactors on infertility has progressed significantly. Based on previous studies, germ cells, anatomic and hormonal disorders, and genetic abnormalities can be considered reasons for infertility in males (32). Sperm abnormalities can emerge in different forms, such as azoospermia (seminal fluid with no sperm), oligospermia (a low concentration of sperm), asthenospermia (low sperm motility), teratozoospermia (decreased sperms with normal morphology), or a combination of them (33).

The motility level is inversely and directly related to *P53* and *SPATA18* expression, respectively. Accordingly, low motility results in increased and decreased *P53* and *SPATA18* gene expression, respectively. Low motility hinders sperm motility, and *P53* prevents *SPATA18* activation and directs cells toward cell death by increasing their expression.

Thus, inactivating *SPATA18* and increasing *P53* expression during infertility can probably intensify the condition.

Male *P53*^{-/-} knockout mice in Zalzal et al.'s (34) study showed a decreased sperm count and abnormal sperm motility and morphology. Their results implicated the central role of cell cycle gene *P53* in some events like sperm development and differentiation. However, unknown pathways and the absence of probable known downstream effectors like *Cdkn1a* highlight the complicated roles of these genes in sperm biology. Nakamura and Arakawa (35) examined the *SPATA18* role in mice fertility. They found that although the *SPATA18*^{-/-} knockout mice were fertile in vivo, the sperm of these mice was severely impaired *in vitro* because of sperm motility failure and the oxidized proteins were dramatically accumulated in the midpiece of *SPATA18*^{-/-} sperms.

Several studies have been conducted on *P53* and *SPATA18* gene expression, although their results are consistent and inconsistent with those of the present study. Other study declared that *SPATA18* expression was reduced by the direct effect of the *P53* regulatory response. However, almost all the other studies reported that this relationship was inverse (14, 30, 36-40).

After surveying the literature to find out the underlying hypothetical mechanism, the model presented by Kitamura et al. (14) was used to interpret the results. Based on the model, increasing *P53* expression and decreasing *Mieap* resulted in inducing *Mieap* by *P53*. *Mieap* functions by two methods. Increasing disturbance in mitochondria, they continued with lysosomes by destroying damaged mitochondria or repairing and returning mitochondria to their natural activity. Further, since knocking down the *Mieap* gene disturbs repairing, an increase in *P53* and a decrease in *SPATA18* in these sperms disturb repair of mitochondria. Consequently, destroying mitochondria leads to the reduction of sperm energy and low motility.

According to Moradi et al. (36), the *P53* level increased significantly in asthenospermic samples. The present study results demonstrated that the *P53* and *SPATA18* gene expression levels increased and decreased in the asthenospermic samples, respectively. However, in their study the ROS level in sperm samples and the activity of thioredoxin reductase (TrxR) and identified sperm DNA fragmentation were assessed by using TUNEL assay, while the SDFa kit was used in the present study to assess the DNA fragmentation level.

Ghandehari-Alavijeh et al. (37) provided a model in which hypoxia markers such as *P53* were significant in infertile males due to asthenospermia compared to fertile ones. Moreover, its high expression, such as *P53*, led to apoptosis, which is in line with the present study results showing that *P53* in the asthenospermic samples was high and directed sperm cells toward apoptosis. However, Ghandehari-Alavijeh et al. (37) assessed reactive oxygen species (ROC) in sperm cells, while the SDFa kit was used in the present study to evaluate the DNA fragmentation level.

Furthermore, considering the data obtained by comparing the sperms without and with DNA fragmentation, the *P53* and *SPATA18* expression levels decreased and increased, respectively, although the difference was insignificant.

Belloc et al. (39) reported that the decomposed fragmented sperm DNA level was more in males with asthenospermia (sperm motility defect) than in males with oligozoospermia or teratozoospermia, which is related to motility. Thus, their motility was low, and consequently, their *P53* and *SPATA18* gene expression levels increased and decreased, respectively.

Conclusion

Based on the results, low motility hinders sperm motility and *P53* prevents *SPATA18* from activation and directs cell toward DNA breaking by increasing its expression, which is considered the apoptosis background. Accordingly, *SPATA18* inactivation and increased *P53* expression during infertility can probably intensify the condition. In other words, the motility level is directly and inversely related to *SPATA18* and *P53* expression, respectively. Thus, lower motility leads to increased and decreased *P53* and *SPATA18* gene expression, respectively. The viability and motility of sperm samples can be found using the gene expression panel of sperms in the future.

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Authors' Contributions

A.P.; Methodology, formal analysis, investigation, resources, and writing (original draft and editing). S.M.A.; Methodology, conceptualization, and supervision. G.A.T.; Formal analysis and resources. All authors read and approved the final manuscript.

References

- Georgiadis AP, Kishore A, Zorrilla M, Jaffe TM, Sanfilippo JS, Volk E, et al. High quality RNA in semen and sperm: isolation, analysis and potential application in clinical testing. *J Urol.* 2015; 193(1): 352-359.
- World Health Organization. WHO Laboratory manual for the examination and processing of human semen. 5th ed. 2010. Available from: <https://apps.who.int/iris/handle/10665/44261> (10 May 2010).
- Araoye MO. Epidemiology of infertility: social problems of the infertile couples. *West Afr J Med.* 2003; 22(2): 190-196.
- Bablok L, Dziadecki W, Szymusik I, Wolczynski S, Kurzawa R, Pawelczyk L, et al. Patterns of infertility in Poland - multicenter study. *Neuro Endocrinol Lett.* 2011; 32(6): 799-804.
- 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.
- Inhorn MC. Middle Eastern masculinities in the age of new reproductive technologies: male infertility and stigma in Egypt and Lebanon. *Med Anthropol Q.* 2004; 18(2): 162-182.
- Jungwirth A, Giwercman A, Tournaye H, Diemer T, Kopa Z, Dohle G, et al. European association of urology guidelines on male infertility: the 2012 update. *Eur Urol.* 2012; 62(2): 324-332.
- Ortega C, Verheyen G, Raick D, Camus M, Devroey P, Tournaye H. Absolute asthenozoospermia and ICSI: what are the options? *Hum Reprod Update.* 2011; 17(5): 684-692.
- Sanocka D, Kurpisz M. Infertility in Poland--present status, reasons and prognosis as a reflection of central and eastern Europe problems with reproduction. *Med Sci Monit.* 2003; 9(3): SR16-SR20.
- Krausz C, Riera-Escamilla A. Genetics of male infertility. *Nat Rev Urol.*

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- 2018; 15(6): 369-384.
11. Brugh VM 3rd, Lipshultz LI. Male factor infertility: evaluation and management. *Med Clin North Am.* 2004; 88(2): 367-385.
 12. Hirsh A. Male subfertility. *BMJ.* 2003; 327(7416): 669-672.
 13. Brugh VM 3rd, Matschke HM, Lipshultz LI. Male factor infertility. *Endocrinol Metab Clin North Am.* 2003; 32(3): 689-707.
 14. Kitamura N, Nakamura Y, Miyamoto Y, Miyamoto T, Kabu K, Yoshida M, et al. Mieap, a p53-inducible protein, controls mitochondrial quality by repairing or eliminating unhealthy mitochondria. *PLoS One.* 2011; 6(1): e16060.
 15. Miyamoto Y, Kitamura N, Nakamura Y, Futamura M, Miyamoto T, Yoshida M, et al. Possible existence of lysosome-like organella within mitochondria and its role in mitochondrial quality control. *PLoS One.* 2011; 6(1): e16054.
 16. Dan X, Babbar M, Moore A, Wechter N, Tian J, Mohanty JG, et al. DNA damage invokes mitophagy through a pathway involving Spata18. *Nucleic Acids Res.* 2020; 48(12): 6611-6623.
 17. Hill S, Van Remmen H. Mitochondrial stress signaling in longevity: a new role for mitochondrial function in aging. *Redox Biol.* 2014; 2: 936-944.
 18. Roberts RF, Tang MY, Fon EA, Durcan TM. Defending the mitochondria: The pathways of mitophagy and mitochondrial-derived vesicles. *Int J Biochem Cell Biol.* 2016; 79: 427-436.
 19. Ashrafi G, Schwarz TL. The pathways of mitophagy for quality control and clearance of mitochondria. *Cell Death Differ.* 2013; 20(1): 31-42.
 20. Martinez-Vicente M. Neuronal mitophagy in neurodegenerative diseases. *Front Mol Neurosci.* 2017; 10: 64.
 21. Okuyama K, Kitajima Y, Egawa N, Kitagawa H, Ito K, Aishima S, et al. Mieap-induced accumulation of lysosomes within mitochondria (MALM) regulates gastric cancer cell invasion under hypoxia by suppressing reactive oxygen species accumulation. *Sci Rep.* 2019; 9(1): 2822.
 22. Prasad S, Gupta SC, Tyagi AK. Reactive oxygen species (ROS) and cancer: Role of antioxidative nutraceuticals. *Cancer Lett.* 2017; 387: 95-105.
 23. Georgieva E, Ivanova D, Zhelev Z, Bakalova R, Gulubova M, Aoki I. Mitochondrial dysfunction and redox imbalance as a diagnostic marker of "free radical diseases". *Anticancer Res.* 2017; 37(10): 5373-5381.
 24. Saleh RA, Agarwal A. Oxidative stress and male infertility: from research bench to clinical practice. *J Androl.* 2002; 23(6): 737-752.
 25. Xu Y, Phoon CK, Berno B, D'Souza K, Hoedt E, Zhang G, et al. Loss of protein association causes cardiolipin degradation in Barth syndrome. *Nat Chem Biol.* 2016; 12(8): 641-647.
 26. Lu YW, Claypool SM. Disorders of phospholipid metabolism: an emerging class of mitochondrial disease due to defects in nuclear genes. *Front Genet.* 2015; 6: 3.
 27. Ren M, Xu Y, Erdjument-Bromage H, Donelian A, Phoon CKL, Terada N, et al. Extramitochondrial cardiolipin suggests a novel function of mitochondria in spermatogenesis. *J Cell Biol.* 2019; 218(5): 1491-1502.
 28. Cohen-Bacrie P, Belloc S, Ménézo YJ, Clement P, Hamidi J, Benkhalifa M. Correlation between DNA damage and sperm parameters: a prospective study of 1,633 patients. *Fertil Steril.* 2009; 91(5): 1801-1805.
 29. Kaneko T, Murayama E, Kurio H, Yamaguchi A, Iida H. Characterization of Spetex-1, a new component of satellite fibrils associated with outer dense fibers in the middle piece of rodent sperm flagella. *Mol Reprod Dev.* 2010; 77(4): 363-372.
 30. Bornstein C, Brosh R, Molchadsky A, Madar S, Kogan-Sakin I, Goldstein I, et al. SPATA18, a spermatogenesis-associated gene, is a novel transcriptional target of p53 and p63. *Mol Cell Biol.* 2011; 31(8): 1679-1689.
 31. Amann RP, Waberski D. Computer-assisted sperm analysis (CASA): capabilities and potential developments. *Theriogenology.* 2014; 81(1): 5-17. e1-3.
 32. Ross C, Morriss A, Khairy M, Khalaf Y, Braude P, Coomarasamy A, et al. A systematic review of the effect of oral antioxidants on male infertility. *Reprod Biomed Online.* 2010; 20(6): 711-723.
 33. Spanò M, Bonde JP, Hjøllund HI, Kolstad HA, Cordelli E, Leter G. Sperm chromatin damage impairs human fertility. The Danish First Pregnancy Planner Study Team. *Fertil Steril.* 2000; 73(1): 43-50.
 34. Zalzal H, Rabeh W, Najjar O, Abi Ammar R, Harajly M, Saab R. Interplay between p53 and Ink4c in spermatogenesis and fertility. *Cell Cycle.* 2018; 17(5): 643-651.
 35. Nakamura Y, Arakawa H. Discovery of Mieap-regulated mitochondrial quality control as a new function of tumor suppressor p53. *Cancer Sci.* 2017; 108(5): 809-817.
 36. Moradi MN, Karimi J, Khodadadi I, Amiri I, Karami M, Saidijam M, et al. Evaluation of the p53 and thioredoxin reductase in sperm from asthenozoospermic males in comparison to normozoospermic males. *Free Radic Biol Med.* 2018; 116: 123-128.
 37. Ghandehari-Alavijeh R, Zohrabi D, Tavalae M, Nasr-Esfahani MH. Association between expression of TNF- α , P53 and HIF1 α with asthenozoospermia. *Hum Fertil (Camb).* 2019; 22(2): 145-151.
 38. Nakamura Y, Kitamura N, Shinogi D, Yoshida M, Goda O, Murai R, et al. BNIP3 and NIX mediate Mieap-induced accumulation of lysosomal proteins within mitochondria. *PLoS One.* 2012; 7(1): e30767.
 39. Belloc S, Benkhalifa M, Cohen-Bacrie M, Dalleac A, Chahine H, Amar E, et al. Which isolated sperm abnormality is most related to sperm DNA damage in men presenting for infertility evaluation. *J Assist Reprod Genet.* 2014; 31(5): 527-532.
 40. Iida H, Honda Y, Matsuyama T, Shibata Y, Inai T. Spetex-1: a new component in the middle piece of flagellum in rodent spermatozoa. *Mol Reprod Dev.* 2006; 73(3): 342-349.