

Sperm dynein ATPase and ATPases associated with various cellular activities (AAA⁺): regulation in oligo-astheno-teratozoospermia and necrozoospermia as extreme sperm motility disorders

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Abstract

Asthenozoospermia is the most frequent sperm motility disorder, but there are other more extreme sperm motility disorders, namely oligo-astheno-teratozoospermia (OAT) and necrozoospermia. There are several cellular mechanisms known for OAT and necrozoospermia, but there are limited data on dynein ATPase and ATPases associated with various cellular activities (AAA⁺).

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AAA1 is involved in ATP hydrolysis, while AAA2 is entangled in ATP-binding pocket. This study was conducted to investigate the role of dynein ATPase activity and quantification of AAA dynein. Spermatozoa from 14 men with OAT, 11 men with necrozoospermic and 17 men with normozoospermic samples were used in this study. Makler chamber was used to determine sperm concentration and motility, while Papanicolaou stained semen smears using World Health Organization-fifth edition criteria was performed to determine sperm morphology, and dynein ATPase was quantified by calculation of released inorganic phosphate. AAA was quantified by enzyme-linked immunosorbent assay, whereas the distribution was determined by immunocytochemistry. This study showed that the dynein ATPase activity in OAT and necrozoospermia was lower than in the normozoospermic group (2.68 ± 0.76 , 1.01 ± 0.31 , 7.22 ± 1.08 $\mu\text{mol Pi/mg protein/h}$, respectively, $P < 0.05$), as well as the amounts of AAA1 and AAA2. In addition, staining for AAA in the sperm tail paralleled the dynein ATPase activity and quantity of AAA, being the highest in sperm from normozoospermic samples, lower in sperm from OAT samples, and almost undetectable in sperm from necrozoospermic samples.

The structure and function of damaged sperm dynein may alter dynein ATPase activity and levels of AAA1 and AAA2.

Introduction

The axonemal structure of the sperm tail plays an important role in sperm motility.¹ The axoneme consists of nine peripheral microtubules doublets with two single central microtubules (9+2), which are composed of dynein.^{2,3} Moreover, dynein comprises one outer arm and one inner arm, which permits sliding of the microtubules resulting in sperm motility.⁴ Therefore, dynein is a protein motor in the sperm axoneme.^{1,4}

Furthermore, the heavy chain structure of dynein is responsible for all the motor activities, namely ATP hydrolysis (dynein ATPase), ATP-sensitive microtubule binding and microtubule shifts. In other words, dynein ATPase is an enzyme confined to the dynein arms of the axonemal microtubules.^{5,6} Dynein uses energy from the hydrolysis of ATP for axonemal beating. Besides the dynein ATPase, there are ATPases associated with various cellular activities (AAA⁺) which are assembled in a ring structure at the head of dynein. There are six domains of AAA⁺, namely AAA1-AAA4 which contain nucleotide binding sites and AAA5-6 which contain fewer sites.^{7,8} According to a study by Kardon, AAA1 is mostly involved in axoneme – sperm motility as an ATP hydrolysis site, whereas AAA2 supports sperm motility by providing the structure for an ATP-binding pocket.⁹ Abnormalities in sperm motility are male factors

responsible for some cases of male infertility.¹⁰ Besides asthenozoospermia as the most frequent abnormality in sperm motility, other more severe and rare abnormalities are oligo-astheno-teratozoospermia (OAT) and necrozoospermia. There are several known cellular mechanisms that cause OAT and necrozoospermia such as ultrastructural defects of the axoneme, centriolar abnormalities, mitochondrial defects, abnormal antioxidant systems, various genetic defects, *etc.*, but there are still limited data about the dynein ATPase and its AAA+. In this study, the main interest was to investigate the role of dynein ATPase activity and dynein AAA1-2 quantification in severe male factor infertility.

Materials and Methods

Semen collection and analysis

Approval from the Ethics Committee of the Faculty of Medicine, University of Indonesia, was obtained before this study began. Semen samples were obtained after an abstinence of 3-5 days. After semen was gained from masturbation and liquified, semen analysis was performed by using Makler chamber for sperm concentration and motility, and Papanicolaou staining for sperm morphology. OAT is defined as all three sperm parameters being below the lower reference values: <15 million/mL for sperm concentration, <40% for progressive motility and <4% for normal sperm morphology, while necrozoospermia is determined if more than 60% of spermatozoa in semen are dead or immotile.¹¹⁻¹³ In this study, the necrozoospermic samples were 100% immotile. The eosin Y staining (0.5% wt/vol) was implemented to assess viability of sperms, then the viable sperms (unstained) were counted in percentage.

Isolation of the sperm axonemal fraction

Modification of the Olson method was applied to isolate the axonemal fraction of spermatozoa.¹⁴ Centrifuging at 1,000 xg for 10 min at 4°C was performed to centrifuge semen diluted in Olson A solution (0.05 M Tris-HCl; 0.1 M NaCl, pH 7.4). After that, centrifuging the pellet in Olson B solution (10 mM Tris-HCl; 0.5 mM EDTA, pH 7.4; protease inhibitor cocktail composed of 0.5 mM phenylmethylsulfonyl fluoride, 5 mM benzamide, 1 µg/mL leupeptin, 1 µg/mL pepstatin A) at 1,000 xg for 10 min at 4°C was performed. An Ultra Turrax homogenizer at 13,000 rpm for 5 s was used to homogenize the supernatant obtained. Then, the suspension was placed on a gradient of sucrose solution (20% (w/v) sucrose with 25 mmol/L Tris-HCl (pH 7.4) and 50% (w/v) sucrose with 25 mmol/L Tris-HCl in pH 7.4). After a final centrifugation at 17,000 xg for 30 min the obtained pellet was added to Olson B solution. 2% (w/v) sodium dodecyl sulfate and 1% (w/v) bovine serum albumin were added to the pellet to remove the membrane component from the axonemal fraction. The protein content of the axonemes was determined by the Bio-Rad micromethod of Bradford¹⁵ before further analysis.

Dynein ATPase activity assay

The Vivenes method was implemented to define dynein ATPase activity.⁴ A solution (5 mmol/L MgCl₂, Tris-base, 10 mM ouabain and 1 µM thapsigargin and 1 mM vanadate) in 9 mL was mixed with 1 mL of the axoneme fraction. Then, 3 mmol/L Na₂ATP was added to start the ATPase reaction and 1 mL 15% (w/v) trichloroacetic acid was added later to terminate this process. Centrifugation at 1100 xg for 10 min produced the pellets and then a colorimetric method against KH₂PO₄ standards was used to

determine released inorganic phosphate (Pi) from the pellet.¹⁶ Finally, the Pi content in the two reactions with and without 1mM vanadate was defined as dynein ATPase activity. Vanadate was added as a dynein ATPase inhibitor, whereas ouabain and thapsigargin were used as the inhibitor of Na⁺, K⁺-ATPase and Ca²⁺-ATPase enzymes.^{4,17,18}

Quantification of AAA protein

Quantification of AAA1 in spermatozoa was performed by using an enzyme-linked immunosorbent assay (ELISA) kit sandwich (Human Dynein Heavy Chain 1, Axonemal (DNAH1) ELISA Kit MBS928073, My Bio Source, San Diego, California, USA. Available from: https://www.mybiosource.com/prods/ELISA-Kit/Human/dynein-axonemal-heavy-chain-1/DNAH1/datasheet.php?products_id=928073) which can measure the AAA1 concentration at 23.5–1500 pg/mL. Various concentrations (1500, 750, 375, 187.5, 94, 47 and 23.5 pg/mL) of standard solutions were prepared first. Protein quantification of the samples was adjusted to 15 µg in 100 mL of samples. Then, the wells were covered with specific monoclonal antibody to AAA1, which was continued by the addition of 100 µL standard solution and 100 µL sample. After that, the solution was put in into wells which then kept at 37°C for 2 h. After removing the solution, 100 µL biotin antibody was placed in the wells, and incubated at 37°C for 1 h. Next, the plate was rinsed with buffer four times and held for 2 min. Streptavidin-Horseradish Peroxidase was added to the wells and incubated at 37°C for 1 h. Then, tetramethyl-benzidine (TMB substrate) was added to the wells and incubated at 37°C for 30 min. The reaction was stopped by 50 µL Stop Solution and the absorbance measured in an ELISA reader at a wavelength of 450 nm. AAA1 quantification was then defined by comparing the optical density (OD) value of the sample with the standard curve. Similar to those of AAA1, the AAA2 levels were examined by ELISA kit (Human Dynein Heavy Chain 2, Axonemal (DNAH2) ELISA Kit MBS9342693, My Bio Source, San Diego, California, USA) which can detect AAA2 levels in the concentration range 3.12-100 ng/mL.

Immunocytochemistry of AAA isoform

Spermatozoa in semen were centrifuged first at 2,407 xg for 10 min before immunocytochemistry. The pellet obtained was mixed with Biggers, Whitten and Whittingham medium after the supernatant was discarded. Another centrifugation at 2,407 xg for 5 min followed. 0.2% (v/v) Triton X-100 and the poly-L-lysine-coated slides with 4% (v/v) formaldehyde were prepared for permeabilization and fixation, respectively. DNAH1 and DNAH2 goat polyclonal antibodies (Santa Cruz, San Diego, California, USA) as primary antibodies were prepared at 1:200 dilution at 4°C overnight for incubation, while donkey anti-goat Ig G antibody as secondary antibody was applied to fluorescein isothiocyanate (Santa Cruz) to define reactions. Detection of the AAA1 and AAA2 signals obtained were analyzed in a confocal microscope (Zeiss, Oberkochen, Germany).

Statistical analysis

In this study, the 22nd version of SPSS was conducted to analyze data. The Kruskal-Wallis test was implemented to analyze differences between dynein ATPase activity and quantification of AAA proteins of OAT and necrozoospermia compared with normozoospermia group. P<0.05 was considered statistically significant.

Results

Semen analysis profile

The semen profiles of this study are shown in Table 1.

Activity of dynein ATPase

The dynein ATPase activity was significantly lower in the OAT and necrozoospermic groups than in the control (normozoospermic group), and the necrozoospermic group showed the lowest activity of dynein ATPase ($P < 0.05$, Table 2). This study showed a similar trend with Na^+ , K^+ -ATPase and Ca^{2+} -ATPase activities, as dynein ATPase activity was significantly lower in OAT and necrozoospermic than the normozoospermic group.

Quantification of AAA protein

The amount of AAA1 protein was significantly lower in OAT and necrozoospermia than in normozoospermia, and the necrozoospermic group had the lowest amount of AAA1 protein ($P < 0.05$, Table 3). Similar to AAA1, the amount of AAA2 protein was also significantly lower in OAT and necrozoospermia than in normozoospermia, whereas the necrozoospermia group showed the lowest amount of AAA2 protein ($P < 0.05$, Table 3).

Distribution of AAA protein

In the normozoospermic samples, AAA1 was located in the entire sperm tail and weak staining in the sperm head. In contrast, the AAA1 in the OAT samples was weak staining in the sperm tail, and in necrozoospermic samples it was almost undetectable

(Figure 1). In addition, the AAA1 in the OAT samples was weak staining in the sperm head, compared to in the necrozoospermic samples. Similar to the AAA1 protein, the AAA2 was also located in the whole tail and head of sperm in normozoospermic samples, while in OAT samples was located weak staining in the tail of sperm and almost undetectable in the sperm head. Furthermore, in necrozoospermic samples, the AAA2 was almost undetectable in tail of sperm (Figure 2). Uniquely, even though AAA is component of the sperm axoneme, both proteins were also located in the membrane of sperm head.

Discussion

Several studies have proven that the cause of OAT and necrozoospermia are infections and toxic substances, with the underlying cellular mechanisms such as ultrastructural defect of the axoneme, centriole abnormalities, mitochondrial defects, abnormal antioxidant systems, various genetic defects and so on.¹⁹⁻²⁸ Furthermore, our recent previous study demonstrated that there is disruption of Na^+ , K^+ -ATPase, Ca^{2+} -ATPase activities and Na^+ , K^+ -ATPase $\alpha 4$ and PMCA4 isoform expression in OAT and necrozoospermic sperm samples.²⁹ However, there are limited data about the role of dynein ATPase and AAA isoforms in OAT and necrozoospermia, as examples of extreme sperm motility disorders. This is the first study that analyze dynein ATPase activity and AAA protein quantification in OAT and necrozoospermia.

Table 1. Mean values of semen parameters in normozoospermia, oligo-astheno-teratozoospermia (OAT), and necrozoospermia.

Parameter	Normozoospermia (n=17)	OAT (n=14)	Necrozoospermia (n=11)
Semen pH	7.2	7.2	7.0
Semen color	Pearl white	Pearl white	Pearl white
Sperm count (million)	99.4±6.2 ^a	15.4±5.5 ^a	2.5±0 ^a
Sperm motility (%)	62.2±5.1 ^a	21.4±1.3 ^a	0
Normal sperm morphology (%)	18.6±2.5 ^b	1.5±0.2 ^b	5.1±1.2 ^b
Sperm viability (%)	74.2±6.6 ^a	52.5±5.4 ^a	11.2±2.4 ^a

n, number of samples. Values are given as the mean ± standard error of the mean. Values with a in superscript are significantly different, while those with b in superscript are not significantly different; $P < 0.05$.

Table 2. Activity of dynein ATPase in the normozoospermic, oligo-astheno-teratozoospermia (OAT) and necrozoospermic groups.

ATPase activity ($\mu\text{mol Pi/mg protein/h}$)	Normozoospermia (n=17)	OAT (n=14)	Necrozoospermia (n=11)
Dynein ATPase	7.2±1.1 ^a	2.7±0.8 ^a	1.0±0.3 ^a

Values are given as the mean ± SEM; values with the same superscript are significantly different; $P < 0.05$.

Table 3. The sperm protein concentration of the normozoospermic, oligo-astheno-teratozoospermic (OAT) and necrozoospermic groups.

Protein concentration (ng/mL)	Normozoospermia	OAT	Necrozoospermia
AAA1	5.2±0.5 ^a	3.7±0.5 ^a	1.9±0.2 ^a
AAA2	4.2±0.6 ^a	2.3±0.53 ^a	2.0±0.6 ^a

Values are given as mean ± SEM; values with the same superscript are significantly different; $P < 0.05$.

Activity of dynein ATPase

Dynein ATPase located in the dynein arms of the sperm microtubule doublet acts by hydrolyzing ATP to slide adjacent axonemal microtubules and generate sperm motility. In this study, dynein ATPase activity was significantly lower in OAT and necrozoospermia than in normozoospermia, whereas the necrozoospermic group showed the lowest dynein ATPase activity. This study confirms the results of Vivenes *et al.*, who proved that dynein ATPase activity in asthenozoospermia was lower than in the normozoospermic group.⁴ In addition, this study also agrees with levels of other ATPases in our previous study, such as Na⁺, K⁺-ATPase and Ca²⁺-ATPase activities in the same group (OAT and necrozoospermia), although it is contrast to Ca²⁺-ATPase activity in the asthenozoospermia group.^{29,30} Na⁺, K⁺-ATPase in the sperm membrane permits influx of Na⁺ and efflux of K⁺, which maintains ion homeostasis and cell surface tension. It seems that Na⁺, K⁺-ATPase and dynein ATPase work together in similar way for sperm viability. On the other hand, Ca²⁺-ATPase works differently by exporting intracellular Ca²⁺, which maintains intracellular Ca²⁺ level to prevent cell toxicity.

In OAT, there are three sperm disorders, namely lower sperm count, reduced sperm motility and abnormal morphology. Sperm morphogenesis especially ultrastructure occurs in a crucial phase in the spermatogenesis process, namely spermiogenesis, so the abnormality of ultrastructure is a consequence of a disruption in spermiogenesis.^{31,32} There are hundreds of genes coding for sperm synthesis, including microtubules with dynein.³³ An example is immotile-cilia syndrome, which is characterized by disruptions of microtubules, such as the absence of dynein arms or the 9+0 axonemal microtubule. The ultimate diagnosis is by electron microscopy, which can reveal the absence of dynein arm or nine outer microtubules completed with dynein arm but lacking the two central microtubules.³⁴

Sperm disorders in OAT are recognized as the etiology of reactive oxygen species (ROS) production in sperm cells.³⁵ If the ROS level is present in high amounts, it will cause oxidative stress.²³ Spermatozoa in OAT are also involved in oxidative stress in spermatozoa.³⁶⁻³⁸ The more severe the disorder, the more the damage

to spermatozoa from ROS production. This phenomenon could explain why dynein ATPase activity in OAT is lower than normozoospermia.

In necrozoospermia, the disorder is more severe than in OAT since there are both sperm motility and viability disorders. Nevertheless, Talebi showed that there is damage of many sperm organelles such as plasma membrane, acrosome, mitochondrial membranes and microtubules in necrozoospermia.³⁹ Necrozoospermia can be caused by infection (male accessory-gland infection, prostatitis, epididymitis), antisperm antibodies, retarded ejaculation, testicular carcinoma, old age and exposure of toxins.⁴⁰ It is essential to classify the source of necrozoospermia and to manage treatment by performing the history taking, urogenital examination, semen analysis, urine culture, antisperm antibody test, transrectal ultrasonography and testicular biopsy.⁴¹ If the source of necrozoospermia is the epididymis, the cause is an inimical milieu or innate disruption of ultrastructure of sperm.²⁸ In this situation, ROS can infiltrate into the sperm cell and generate deteriorating processes in nucleus producing DNA damage.⁴² In addition, other authors have assumed the apoptotic process in spermatozoa is related their prolonged sojourn in male genital tract.⁴¹

Again, the more severe the disorder, the more damaged the spermatozoa are. This phenomenon may explain why in necrozoospermia dynein ATPase activity is lower than in OAT. This is the first study demonstrating the declining of dynein ATPase activity in sperm motility disorder, especially in OAT and necrozoospermia.

Quantification of AAA protein

This study is the first to show a decline on AAA in OAT and necrozoospermia. The results of this study are in line with the Na⁺, K⁺-ATPase $\alpha 4$ isoform and PMCA4 expression in OAT and necrozoospermia.³⁰ They are also in accordance with the Na⁺, K⁺-ATPase $\alpha 4$ expression, which is crucial for ion homeostasis for cell surface tension, and PMCA4 expression, which is important for maintaining Ca²⁺ level in cell.⁴³⁻⁴⁵ Besides membrane disruption, there is also dynein disruption in sperm motility disorder. The result of this study confirmed that there was alteration in AAA

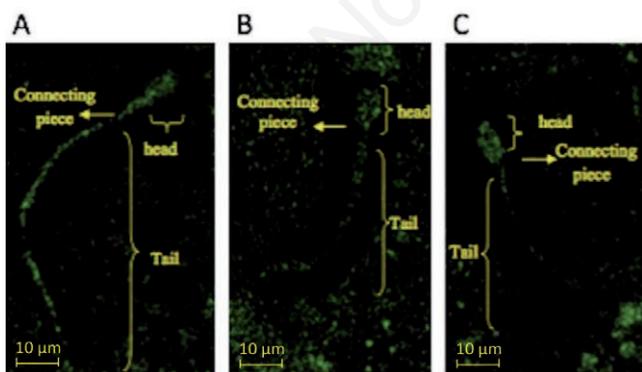


Figure 1. Immunocytochemical analysis shows the localization of AAA1 isoforms in spermatozoa. A) AAA1 isoform is located in the whole sperm (head and tail) in normozoospermic samples; B) AAA1 isoform in oligo-astheno-teratozoospermic samples was weak staining in the sperm head and tail compared to necrozoospermic and normozoospermic samples, respectively; C) AAA1 isoform in necrozoospermic samples is almost undetectable in the sperm tail.

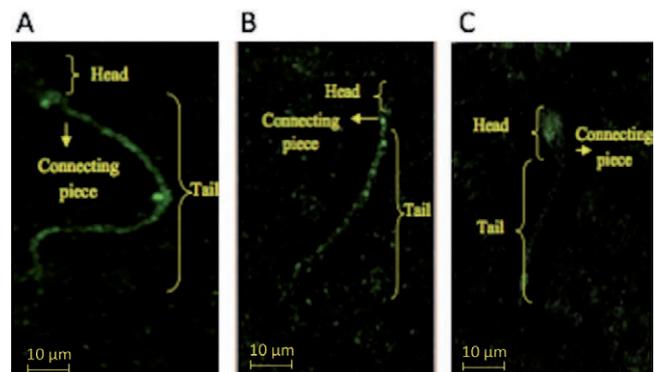


Figure 2. Immunocytochemical analysis shows the localization of AAA2 isoforms in spermatozoa. A) The AAA2 isoform is located in the whole sperm tail in normozoospermia; B) the AAA2 isoform in oligo-astheno-teratozoospermic samples is weak staining in the sperm head and tail compared to necrozoospermia and normozoospermic samples, respectively; C) the AAA2 isoform in necrozoospermic samples is almost undetectable in the sperm tail, even though it still present in the sperm head.

quantity in the pathological spermatozoa in OAT and necrozoospermia. In addition, our other study also shows that spermatozoa after preparation to select more viable and motile cells expressed more AAA than spermatozoa in whole semen or before preparation.¹⁵

Distribution of AAA isoform

This is the first study that measures the expression of AAA protein in spermatozoa with extreme motility disorders. There was less expression or distribution of AAA1 and AAA2 protein in the tail of spermatozoon, whereas the distribution in necrozoospermia was less than in OAT. These results are in line with the Na⁺, K⁺-ATPase α 4 and PMCA4 expression in the membrane of sperm head.³⁰ Nevertheless, there were also unique results of both studies, since the expression of AAA was compact in the tail of sperm compared to sperm head, while the expression of Na⁺, K⁺-ATPase α 4 and PMCA4 was compact in the head of sperm compared to in membrane of sperm head. Finally, disturbances in dynein ATPase activity and AAA quantification/expression may impair the sliding mechanism of flagellum, involved in sperm motility. Dynein ATPase activity and AAA expression data might be an indicator of a sperm motility disorder.

Conclusions

The damaged sperm dynein structure and function may alter the dynein ATPase activity and the quantification of AAA1 and AAA2, therefore it will lead to sperm motility disorder. Moreover, additional studies are necessary to expose the other cellular regulation in sperm motility.

References

- Inaba K. Molecular architecture of the sperm flagella: Molecules for motility and signaling. *Zool Sci* 2003;20:1044-56.
- Lindemann C. Struktura-functional relationships of the dynein, spoked and central-pair projections predicted from an analysis of the forces acting within a flagellum. *Biophys J* 2003;84:4114-26.
- Afzelius B. Electron microscopy of the sperm tail. Results obtained with a new fixative. *J Biophys Biochem Cytol* 1959;5:269.
- Vivenes CY, Peralta-Arias RD, Camejo MI, et al. Biochemical identification of dynein-ATPase activity in human sperm. *Z Naturforsch C* 2009;64:747-53.
- Cho C, Vale RD. The mechanism of dynein motility: insight from crystal structures of the motor domain. *Biochim Biophys Acta* 2012;1823:182-91.
- Linck RW, Chemes H, Albertini DF. The axoneme: the propulsive engine of spermatozoa and cilia and associated ciliopathies leading to infertility. *J Assist Reprod Genet* 2016;33:141-1156.
- Koonce M, Samso M. Overexpression of cytoplasmic dynein's globular head causes a collapse of the interphase microtubule network in *Dictyostelium*. *Mol Biol Cell* 1996;7:935-48.
- King SM. The dynein microtubule motor. *Biochim Biophys Acta* 2000;1496:60-75.
- Kardon J, Vale R. Regulators of the cytoplasmic dynein motor. *Nat Rev Mol Cell Biol* 2009;10:854-65.
- Gurfinkel E, Cedenho AP, Yamamura Y, Srougi M. Effects of acupuncture and moxa treatment in patients with semen abnormalities. *Asian J Androl* 2003;5:345-8.
- World Health Organization. WHO laboratory manual for the examination and processing of human semen, 5th ed. Geneva, Switzerland: World Health Organization Press; 2010.
- Merriam-Webster. Merriam-Webster's Medical Dictionary. Springfield, Massachusetts, USA: Merriam-Webster; 2016.
- Fang S, Baker HW. Male infertility and adult polycystic kidney disease are associated with necrozoospermia. *Fertil Steril* 2003;79:643-4.
- Olson GE, Winfrey VP, Garbers DL, Noland TD. Isolation and characterization of a macromolecular complex associated with the outer acrosomal membrane of bovine spermatozoa. *Biol Reprod* 1985;33:761-79.
- Lestari SW, Larasati MD, Mansur IG, et al. Sperm dynein AAA1 and AAA2 expression in human sperm: A regulation in sperm preparation. *Biomed Pharmacol J* 2018;11:77-84.
- Fiske CH, Subbarow Y. The colorimetric determination of phosphorus. *J Biol Chem* 1925;66:375-400.
- Koçak-Toker N, Aktan G, Aykaç-Toker G. The role of Na,K-ATPase in human sperm motility. *Int J Androl* 2002;25:180-5.
- Schuh K, Cartwright EJ, Jankevics E, et al. Plasma membrane Ca²⁺ ATPase 4 is required for sperm motility and male fertility. *J Biol Chem* 2004;279:28220-6.
- Khosrowbeygi A, Zarghami N, Deldar Y. Correlation between sperm quality parameters and seminal plasma antioxidants status. *Iran J Reprod Med* 2004;2:58-64.
- Kumalic IS, Pinter B. Review of clinical trials on effects of oral antioxidants on basic semen and other parameters in idiopathic oligoasthenoteratozoospermia. *Biomed Res Int* 2014;2014:426951.
- Samplaski MK, Dimitromanolakis A, Lo KC, et al. The relationship between sperm viability and DNA fragmentation rates. *Reprod Biol Endocrinol* 2015;13:42.
- Lodhi LA, Zubair M, Qureshi ZI, et al. Correlation between hypo-osmotic swelling test and various conventional semen evaluation parameters in fresh Nili-Ravi buffalo and Sahiwal cow bull semen. *Pakistan Vet J* 2008;28:186-8.
- Agarwal A, Allamaneni SS. Free radicals and male reproduction. *J Indian Med Assoc* 2011;109:184-7.
- Nduwayo L, Barthélémy C, Lansac J, et al. Management of necrozoospermia. *Contracept Fertil Sex* 1995;23:682-5.
- Coutinho EM, Melo JF, Barbosa I, Segal SJ. Antispermatogenic action of gossypol in men. *Fertil Steril* 1984;42:424-30.
- El-Gothamy Z, El-Samahy M. Ultrastructure sperm defects 27.in addicts. *Fertil Steril* 1992;57:699-702.
- Lerda D, Rizzi R. Study of reproductive function in persons occupationally exposed to 2,4- dichlorophenoxyacetic acid (2,4-D). *Mutat Res* 1991;262:47-50.
- Wilton LJ, Temple-Smith PD, Baker HW, de Kretser DM. Human male infertility caused by degeneration and death of sperm in the epididymis. *Fertil Steril* 1988;49:1052-8.
- Lestari SW, Miati DN, Asmarinah. Oligoasthenoteratozoospermia and necrozoospermia: A study of sperm Na⁺, K⁺-ATPase α 4 and plasma membrane Ca²⁺-ATPase regulation. *Online J Biol Sci* 2018;18:304-14.
- Lestari SW, Miati DN, Soeharso P, et al. Sperm Na⁺, K⁺-ATPase α 4 and plasma membrane Ca²⁺-ATPase (PMCA) 4 regulation in asthenozoospermia. *Syst Biol Reprod Med* 2017;63:294-302.
- Zamboni L. The ultrastructural pathology of the spermato-

- zoon as a cause of infertility: the role of electron microscopy in the evaluation of semen quality. *Fertil Steril* 1987;48:711-34.
32. Baccetti B, Burrini A, Capitani S, et al. *Notulae seminologicae*. 1. New combinations of Kartagener's syndrome. *Andrologica* 1993;25:325-9.
 33. Yatsenko AN, Iwamori N, Iwamori T, Matzuk MM. The power of mouse genetics to study spermatogenesis. *J Androl* 2010;31:34-44.
 34. Afzelius BA, Dallai R, Lanzavecchia S, Bellon PL. Flagellar structure in normal human spermatozoa and in spermatozoa that lack dynein arms. *Tissue Cell* 1995;27:241-7.
 35. Dama M, Kaleem AM, Mahdi AA, et al. A case of oligoasthenoteratozoospermia with AZFc deletion and persistent oxidative stress. *Indian J Hum Genet* 2012;18:359-62.
 36. Said TM, Aziz N, Sharma RK, et al. Novel association between sperm deformity index and oxidative stress-induced DNA damage in infertile male patients. *Asian J Androl* 2005;7:121-6.
 37. Kao SH, Chao HT, Chen HW, et al. Increase of oxidative stress in human sperm with lower motility. *Fertil Steril* 2008;89:1183-90.
 38. Pasqualotto FF, Sundaram A, Sharma RK, et al. Semen quality and oxidative stress scores in fertile and infertile patients with varicocele. *Fertil Steril* 2008;89:602-7.
 39. Talebi AR, Khalili MA, Nahangi H, et al. Evaluation of epididymal necrozoospermia following experimental chronic spinal cord injury in rat. *Iran J Reprod Med* 2007;5:171-6.
 40. Lecomte PJ, Barthelemy C, Nduwayo L, Hamamah S. Necrozoospermia: etiology and management. In: Hamamah S, Mieusset R, Olivennes F, Frydman R, eds. *Male sterility and motility disorders*. Proceeding of the International Symposium on Male Sterility for Motility Disorders, sponsored by Serono Symposia USA. Paris: Springer Publishing Group; 1998. pp 65-78.
 41. Ortega C, Verheyen G, Raick D, et al. Absolute asthenozoospermia and ICSI: what are the options? *Hum Reprod Update* 2011;17:684-92.
 42. Agarwal A, Saleh RA. Role of oxidants in male infertility: rationale, significance, and treatment. *Urol Clin North Am* 2002;29:817-27.
 43. Sanchez G, Nguyen ANT, Timmerberg B, et al. The Na, K-ATPase $\alpha 4$ isoform from humans has distinct enzymatic properties and is important for sperm motility. *Mol Hum Reprod* 2006;12:565-6.
 44. Mobasher A, Avila J, C zar-Castellano I, et al. Na⁺, K⁺-ATPase isozyme diversity; comparative biochemistry and physiological implications of novel functional interactions. *Biosci Rep* 2000;20:51-91.
 45. Jimenez T, McDermott JP, S nchez G, Blanco G. Na, K-ATPase $\alpha 4$ isoform is essential for sperm fertility. *Proc Natl Acad Sci USA* 2011;108:644-9.