Asthenozoospermia: Through the eyes of histology and embryology specialist

Histoloji ve embriyoloji uzmanı gözüyle: Asthenozoospermi

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Özet

Sperm motilitesi semen analizinde önemli bir parametredir. Düşük sperm motilitesi olarak tanımlanan asthenozoospermi erkek infertilitesinin önemli nedenlerinden biridir. Asthenozoospermi enfeksiyon, bakteri ve bakteriyel ürünler, sitokinler, anormal sperm gibi pek çok faktörle ilişkili olabilmektedir. Fakat moleküler mekanizması henüz tam olarak aydınlatılamamıştır. İnsan semen örnekleri, DSÖ (Dünya Sağlık Örgütü) kriterlerine göre deneyimli teknisyenler tarafından değerlendirilir. Semen örneği faz kontrast mikroskop altında incelenir ve motilite yüzdesi saptanır. Semen analizi sırasında gözlenen hiperviskozite, azalan sperm motilitesi dolayısıyla erkek infertilitesi ile ilişkilidir. Seminal lökositlerle sperm motilitesi arasındaki ilişki de hala tartışmalıdır. İmmotil spermin canlı olup olmadığını değerlendirmek için canlılık testleri uygulanmaktadır. En sık kullanılan canlılık testleri eozin canlılık testi ve hiperozmolar şişme testi (HOS)' dir. Asthenozoospermi olgularında motilite bozukluğuna sperm morfoloji bozuklukları da eklenince tablo daha da ağırlaşmaktadır. Sperm morfoloji değerlendirilmesinde sık olarak Papanicolaou, Shorr veya Diff-Quik boya yöntemleri kullanılmaktadır. Sonuç olarak basit ve noninvaziv olan semen analizi ile erkek infertilitesine ilişkin pek çok veri elde edilebilmektedir. Doğru ve güvenilir semen analizi ile asthenozoospermi etiyolojisi aydınlatılarak motilite tedavisinde önemli ilerleme kaydedilebilir. Bu derlemede histoloji ve embriyoloji uzmanı gözüyle asthenozoosperminin değerlendirilmesi hedeflenmiştir.

Anahtar Kelimeler: Asthenozoospermi, motilite, sperm, semen analizi

Abstract

Sperm motility is an important parameter in semen analysis. Asthenozoospermia defined as low sperm motility is one of the major causes of male infertility. Asthenozoospermia could be due to many factors such as infection, bacteria and bacterial products, cytokines, abnormal sperm. But molecular mechanism is not fully understood. Human sperm samples are evaluated by trained technicians according to WHO (World Health Organization) criteria. Semen sample is observed under a phase contrast microscope and the motility rate is determined. Hyperviscosity during semen analysis is negatively related to decreased sperm motility and has a negative impact on male infertility. The association between seminal leukocytes and sperm motility is still a matter of debate in the literature. Viability tests should be performed to determine if the immotile sperm are alive or dead. Generally semen samples are subjected to eosin viability test or hypo-osmolar swelling (HOS) test. In cases with asthenozoospermia infertility increases to a greater extent when sperm morphology disorders are added to coexisting motility disorders. Generally in the assessment of sperm morphology are used Papanicolaou, Shorr or Diff-Quik staining methods. As a result, it can be seen that plenty of data are available to provide related to male infertility by means of carrying out basic and not invasive semen analysis. By accurate and confidential semen analysis etiology of asthenozoospermia may be illuminated and important progress can be taken in motility treatment. In this review it is aimed to evaluate asthenozoospermia through the eyes of histology and embryology specialist.

Key Words: Asthenozoospermia, motility, sperm, semen analysis

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Introduction

Sperm motility is an important parameter in semen analysis. Asthenozoospermia defined as low sperm motility is one of the major causes of male infertility.

Asthenozoospermia could be due to many factors such as infection, bacteria and bacterial products, cytokines, abnormal sperm. But molecular mechanism is not fully understood. Extracellular and intracellular ATP (adenosine triphosphate) molecules have critical roles in sperm function. It is the main energy source used by the sperm flagellum and progressive forward motility **(1).** Several endogenous male factors such as antibodies, agglutination factors, donor age and extent of sperm maturation and exogenous factors such as pH, osmolality, viscosity, temperature, ionic composition, hormones, cyclic nucleotides, kinins, prostoglandins and immunologic agents may affect sperm motility **(2).**

Human sperm samples are evaluated by trained technicians according to WHO (World Health Organization) criteria. The updated version of the WHO manual was published in 2010. Standard procedures in semen analysis include evaluation of sperm concentration, motility, vitality and morphology **(3).**

Semen analysis and sperm motility

Basal sperm analysis is obtained from all cases after 2-7 days of sexual abstinence. Sperm motility is determined after about 30 minutes liquefaction period of the samples. In men, WHO has defined the lower reference limit for total motility (progressive motility+ nonprogressive motility) is 40% and the lower reference limit for progressive motility 32 %. Generally Makler Counting Chamber is used to measure sperm motility. Semen sample is observed under a phase contrast microscope and the motility rate is determined. The evaluation may differ between different observers or even with same observer. Also the processing time from semen sampling to examination may affect the result of semen analysis. Another method is a computer aided semen analyzer **(3).**

Asthenozoospermia and hyperviscosity

Freshly ejaculated semen is a coagulum that liquefies over a 20 to 30 minutes period. Maximal duration is 30 to 45 minutes. Viscosity means the stickiness or resistance to flow of the semen. The proteolytic enzymes that initiate liquefaction are found in the prostate and substances secreted by the seminal vesicles coagulate semen. Failure of liquefaction leads to hyperviscous semen **(4).**

In our study we investigated the relation between semen hyperviscosity and low sperm motility count. We detected that hyperviscosity during semen analysis is negatively related to decreased sperm motility and has a negative impact on male infertility **(5).** Low sperm motility which is an important reason for infertility can be recovered by eliminating the hyperviscosity.

Asthenozoospermia and seminal leukocytes

The association between seminal leukocytes and sperm motility is still a matter of debate in the literature. A total 89 male patients, 36 with sperm motility disorder and 53 without any motility disorder, applying to our clinic were included in the study. Semen samples were examined in both macroscopic and microscopic levels. Makler counting chamber was used for the sperm motility and concentration analysis. Leukocytic stain was used for the semen leukocyte analysis **(Figure 1**).

Although in our study we determined no significant correlation between reduced sperm motility and leukospermia, further studies with more patients are needed for semen leukocyte analysis in asthenozoospermic cases which account for a significant proportion of all cases of male infertility **(6).**

Asthenozoospermia and viability tests

Viability tests should be performed to determine if the immotile sperm are alive or dead. Generally semen samples are subjected to eosin viability test or hypo-osmolar swelling (HOS) test. The lower reference limit for vitality is 58% according to WHO criteria. The result of the eosin test is accepted as normal in the case of presence of 58% or more unstainable spermatozoa in a semen sample. The result of HOS test is accepted as normal if swelling behavior is observed in 58% or more of the spermatozoa tails in a semen sample **(3).**

We investigated whether one of the two viability tests is superior to the other in cases with asthenozoospermia in which the rate of immotile spermatozoa exceeded 50%. The binomial (sign test) statistical evaluation revealed that the two tests have no superiority over one another ($p>0,05$). The fact that the HOS test as a viability test yielded very similar results when compared to the eosin test demonstrates the significance of the HOS test in the

sense that it is economic**. (7, Figure 2).**

Asthenozoospermia and sperm morphology dyes

In cases with asthenozoospermia infertility increases to a greater extent when sperm morphology disorders are added to coexisting motility disorders. Generally in the assessment of sperm morphology are used Papanicolaou, Shorr or Diff-Quik staining methods. The lower reference limit for normal forms is 4% **(3).** Usually in IVF (in vitro fertilization) Units sperm morphology is evaluated by Diff-Quik or Spermac (modified Papanicolaou stain) stains. We compared Spermac and Diff-quik methods in the assessment of sperm morphology in asthenozoospermia cases **(Figure 3).** We found that neither of the methods has proven superiority to the other. So a cost reduction may be provided by choosing the economical one **(8)**

Asthenozoospermia and paternal age

It is well-known that maternal age has a negative effect on fertility. Women's fertility peaks at the age of 25. From age 35 woman's fertility drops and by age 40 about one third of women are infertile **(9,10).**

A woman's age is a very important prognostic factor for the success of ART (assisted reproductive technologies). The chance of having a baby decreases after the age 35 and especially after age 40 **(11,12).** It is well known that the success of ICSI (intracytoplasmic sperm injection) is dependent on the woman's age. However, relatively few studies have investigated the effect of advanced paternal age on ICSI outcome **(13,14).**

We evaluated retrospectively the impact of male age on fertilization and embryo quality in couples receiving ICSI. A total of 58 ICSI cycles were included in the study. While semen parameters were displaying serious anomalies in 30 out of the 58 ICSI cycles, semen parameters for the remaining 28 ICSI cycles were selected from subfertile (n=19) and fertile (n=9) group with repeated unsuccessful IVF cycles. Our data demonstrated that male age in our grouping had no effect on fertilization, embryo quality and pregnancy development **(15).** But we think that it needs more studies with more patients about this subject.

Asthenozoospermia and sperm manipulation procedures

A patient with asthenozoospermia can benefit from sperm manipulation procedures and assisted reproductive technology. Asthenozoospermia, particularly severe cases influence the pregnancy success rates following assisted pregnancy techniques. Sperm preparation techniques (concentration, swim-up or gradient methods) differ according to sperm count and motility. Specimens with a good sperm count and motility may be prepared with swim up method. Gradient method is used for specimens with sperm counts less than 5 million/ml and poor motility or having too many scrap cells. Sperm specimens with sperm counts less than 0.5 million/ml and motility less than % 10 is prepared by concentration method **(16).** Semen preparation with pentoxifylline (amethyxanthin derivative) may improve sperm motility. Some studies suggest that pentoxifylline has no effect on the number of progressively motile spermatozoa in normozoospermic

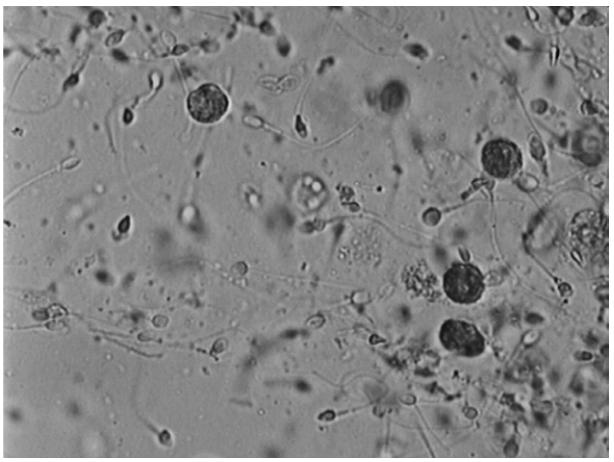

Fig. 1. Leucocytes in semen. Semen sample stained with Leucoscreen (x 400)

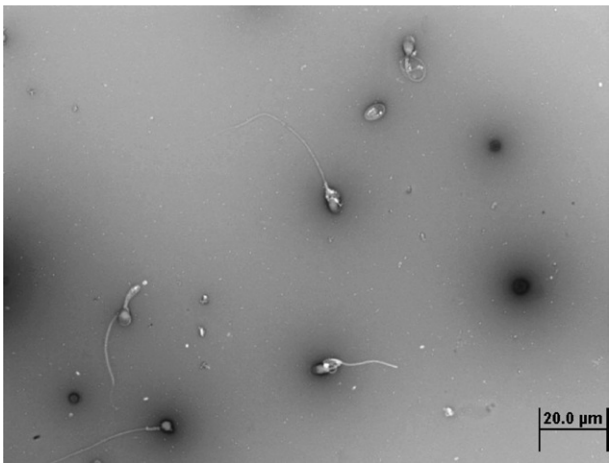

Fig. 2. Died sperms(pink) . Semen sample stained with Eosin (x 400)

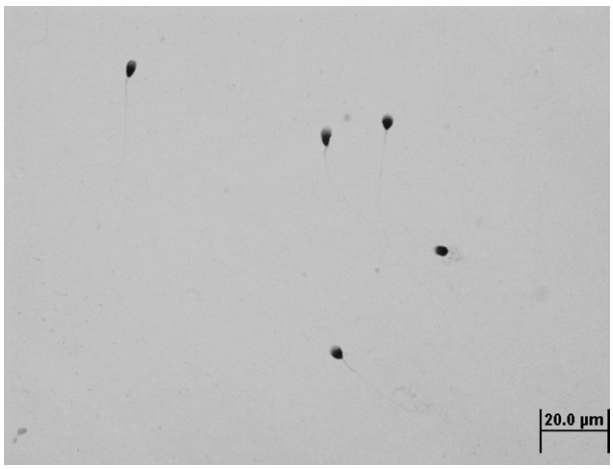

Fig.3. A case of asthenozoospermia: Sperms with head, middle piece and tail defects (Diff-Quik stain, x 1000

samples. But in asthenozoospermic cases it increases the number of progressively motile spermatozoa **(17).**

Conclusion

Hyperviscosity is related to low sperm motility and therefore to male infertility. No superior impact between dyes used for morphological and survival tests led to the usage of economic dyes. Male age in our grouping had no effect on fertilization, embryo quality and pregnancy development. As a result, it can be seen that plenty of data are available to provide related to male infertility by means of carrying out basic and not invasive semen analysis. By accurate and confidential semen analysis etiology of asthenozoospermia may be illuminated and important progress can be taken in motility treatment**.**

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