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Is There a Linear Relationship Between Sperm ATP Levels and Non-Motility of Sperm?

Sperm ATP Düzeyleri ile Sperm Hareketsizliği Arasında Doğrusal Bir İlişki Var Mı?

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Abstract

Background: Asthenozoospermia (AS) and oligoasthenoteratozoospermia (OAT) are characterized by varying degrees of sperm immotility. While ATP deficiency has been proposed as a cause of impaired motility, the relationship between the immotile sperm phenotype and intracellular Adenosine triphosphate (ATP) content remains insufficiently explored. The aim of this study was to evaluate the relationship between sperm motility impairment and intracellular ATP content in AS and OAT.
Materials and Methods: Semen samples were obtained from three groups of men (n = 30 per group): patients diagnosed with OAT, AS, and fertile controls. Semen analysis was performed in accordance with the World Health Organization (WHO) guidelines. Following sperm isolation, intracellular ATP and Adenosine diphosphate (ADP) levels were quantified in sperm cell lysates using a bioluminescence-based assay.
Results: Sperm ATP levels were significantly higher in the OAT group compared with both AS and age-matched controls. Despite elevated ATP levels, no linear correlation was observed between progressive sperm motility and ATP levels in any group. The ADP/ATP ratio was significantly elevated in OAT spermatozoa and showed a strong negative correlation with progressive motility ($r=-0.84$, $p<0.0001$).
Conclusions: The absence of a linear correlation between ATP levels and progressive motility in both AS and OAT groups suggests that reduced motility is unlikely to be the primary determinant of sperm ATP content. The elevated ADP/ATP ratio observed in OAT sperm may reflect an imbalance between ATP production and/or utilization rather than increased ATP availability. Together, these findings highlight the complexity of sperm ATP metabolism and emphasize the importance of evaluating both energy synthesis and energy consumption when assessing sperm function and male infertility.
Keywords: ATP, Asthenozoospermia, Oligoasthenoteratozoospermia, Motility, Sperm

Öz

Amaç: Asthenozoospermi (AS) ve oligoasthenoteratozoospermi (OAT), farklı derecelerde sperm hareketsizliği ile karakterize edilen erkek infertilitesi fenotipleridir. Adenosine triphosphate (ATP) eksikliğinin sperm motilitesinde azalmaya yol açtığı öne sürülmüş olsa da, hareketsiz sperm fenotipinin hücre içi ATP düzeyleri ile nasıl bir ilişki içinde olduğu yeterince araştırılmamıştır. Bu çalı şma, AS ve OAT olgularında sperm hareketsizliği ile ATP düzeyleri arasındaki ilişkiyi değerlendirmeyi amaçlamaktadır.
Materyal ve metod: Semen örnekleri, AT, AS tanılı ve fertil kontrol grubundaki erkeklerden oluşan üç gruptan (her grup n=30) toplanmış ve Dünya Sağlık Örgütü (DSÖ) kriterlerine göre değerlendirilmiştir. Sperm hücreleri ayrıldıktan sonra hücre lizatı içinde ATP ve Adenosine diphosphate (ADP) düzeyleri biyolüminesans yöntemiyle ölçüldü.
Bulgular: OAT grubunda sperm ATP düzeyleri, hem AS hem de yaş-eşleştirilmiş kontrol grubuna göre daha yüksekti. ATP düzeylerindeki artışa rağmen, hiçbir grupta ilerleyici motilite yüzdesi ile ATP düzeyleri arasında anlamlı bir doğrusal ilişki gözlenmedi. OAT spermatozoalarında ADP/ATP oranı anlamlı şekilde yüksek bulundu ve bu oran motilite ile güçlü negatif korelasyon ($r=-0,84$, $p<0,0001$) gösterdi. Bu sonuçlar, motilite ve enerji metabolizmasındaki değişimlerin yalnızca ATP üretimi ile açıklanamayacağını ortaya koymaktadır.
Sonuç: AS ve OAT gruplarında ATP düzeyleri ile ilerleyici motilite arasında doğrudan ilişki olmaması, azalmış motilitenin sperm ATP içeriğini etkileyen tek ya da başlıca faktör olmadığını göstermektedir. OAT grubundaki yüksek ADP/ATP oranı, ATP üretimi ve /veya dengesizlik olduğunu düşündürmektedir. Sperm fonksiyonları ve erkek infertilitesi incelenirken hem enerji sentezi hem de kullanım süreçlerinin birlikte değerlendirilmesi önem arz etmektedir.
Anahtar Kelimeler: ATP, Astenozoospermi, Oligoastenoteratozoospermi, Motilite, Sperm

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Introduction

Adenosine triphosphate (ATP) is essential for spermatozoa, serving as the primary energy source for multiple physiological functions critical to successful fertilization. ATP, primarily generated through glycolysis and oxidative phosphorylation (OXPHOS) (1), is indispensable for sperm maturation, motility, protein modification, and hyperactivation. Notably, sperm motility one of the most defining functional characteristics of spermatozoa relies heavily on ATP, with approximately 70% of cellular ATP devoted to sustaining movement, imposing exceptionally high energetic demands compared to most somatic cells (2).

Numerous studies have investigated ATP production in spermatozoa and its role in sperm pathologies (1,3-6), with many focusing on the relationship between ATP-dependent processes and motility, as well as the mechanisms underlying motility defects (7-9). Despite extensive research on ATP production, the dynamics of ATP consumption in spermatozoa remain poorly characterized.

Evidence from human and animal models suggests that impairments in metabolic pathways including mitochondrial oxidative phosphorylation, glycolysis, the Krebs cycle, fatty acid oxidation, and ketone body oxidation can negatively affect sperm motility and contribute to male infertility (10-14). Given that motility is a major ATP-consuming process, an important yet unresolved question arises: does reduced motility in sperm pathologies lead to increased intracellular ATP levels?

In conditions such as oligoas the no teratozoospermia (OAT) and asthenozoospermia (AS), potential impairments in ATP-consuming pathways beyond motility may generate distinct ATP accumulation patterns. Although existing literature suggests a possible association between sperm ATP levels and motility defects, contradictory findings limit definitive conclusions (7-9,15,16). Most studies assess sperm ATP and motility in a general context, with few investigations distinguishing between specific motility-related pathologies.

The complexity of ATP consumption in spermatozoa, together with diverse pathophysiological mechanisms, introduces confounding factors. Importantly, grouping pathologies such as AS and OAT under a single motility-impaired category may obscure clinically relevant differences, despite the known spectrum of motility impairment ranging from AS to the more severe OAT phenotype (14). Considering the markedly lower motility observed in OAT compared with AS, evaluating the linear relationship between ATP levels and motility separately in these two conditions is essential.

Thus, the aim of this study was to quantify ATP levels in OAT and AS spermatozoa, examine their relationship with motility parameters, and explore the contribution of reduced sperm motility to intracellular ATP content.

Materials and Methods

Study Design, Participants, and Semen Collection

This study included semen samples obtained from 90 voluntary male donors who provided written informed consent. Participants were recruited from the Cerrahpaşa Medical Faculty Andrology Center and categorized into three groups: 30 OAT patients, 30 AS patients, and 30 fertile controls. Participants were aged between 25 and 45 years. Fertile controls had fathered children within the previous two years and exhibited normal semen parameters.

Individuals with malignancy, chronic systemic diseases (including diabetes mellitus, hepatobiliary disorders, and advanced renal failure), chromosomal abnormalities, or endocrine dysfunctions were excluded. Semen analysis was performed according to the World Health Organization (WHO) Laboratory Manual for the Examination and Processing of Human Semen, 6th Edition (2021). Participants maintained a minimum of 72 hours of sexual abstinence before sample collection.

Samples were collected in sterile containers and delivered to the laboratory within one hour. This study was approved by the Cerrahpaşa Faculty of Medicine Clinical Research Ethics Committee (approval no: A-51, date: February 2, 2021).

Definitions of AS and OAT: AS was defined as total motility <40% or progressive motility <32%, while OAT was defined as sperm concentration <15 × 10^6/mL, progressive motility <32%, and normal morphology <4%, in accordance with WHO criteria.

Assessment of Sperm Motility

Sperm motility was assessed using phase-contrast microscopy at 400× magnification. Spermatozoa were categorized as: (a) rapidly progressive, (b) slowly progressive, (c) non-progressive, and (d) immotile. Total motility was calculated as the sum of categories (a), (b), and (c), while progressive motility included categories (a) and (b).

At least 200 spermatozoa per sample were evaluated across 8-10 non-overlapping microscopic fields using a Makler chamber. All measurements were performed in duplicate and independently assessed by two trained observers. Discrepancies exceeding 10% were re-evaluated.

Sperm Morphology (Kruger Morphology)

Spermatozoa were structurally evaluated by examining the head, neck, midpiece, principal piece, and tail regions separately.

Processing of Sperm Samples

A 1 mL aliquot of each semen sample was centrifuged at 1,000 × g for 10 min at 4°C. The pellet was resuspended in 1 mL PBS and centrifuged again at 1,000 × g for 5 min at 4°C. The supernatant was discarded, and 50 µL of 50 mM Tris-HCl (pH 6.8, 4-8°C) was added to the pellet, followed by vortexing and incubation at 4°C for 30 min. After centrifugation (1,000 × g, 10 min, 4°C), the supernatant was removed. For further lysis, 1 µL Proteinase K (20 µg/mL) and 500 µL cell lysis buffer were added, and samples were incubated at 56°C for 15 min with agitation. The samples were then centrifuged at 1,000 × g for 5 min at 4°C. The pellet was resuspended in 750 µL PBS and washed twice by centrifugation (1,000 × g, 7 min, 4°C). Microscopic evaluation confirmed the removal of leukocytes. Processed samples were stored at -80°C until analysis.

Measurement of Intracellular ATP Levels

Following sonication, intracellular ATP levels in sperm suspensions were measured using the ATP Bioluminescence Analysis Kit (CLC II; Roche, Boehringer Mannheim, Germany) according to the manufacturer's protocol. ATP concentrations were determined using a standard calibration curve and normalized to total protein content. Total protein was quantified using the Lowry-Hartree modified method (22,23).

Statistical Analysis

Data were analyzed using analysis of variance (ANOVA) or the nonparametric Kruskal-Wallis test, depending on data distribution. A p value ≤0.05 was considered statistically significant. Spearman's correlation coefficients were calculated to assess relationships between variables. Statistical analyses were performed using GraphPad Prism 5.0 (GraphPad Software, La Jolla, CA, USA).

Results

Compared with the control group, both idiopathic OAT and AS groups exhibited a higher percentage of abnormal sperm morphology and reduced total and progressive motility %. Furthermore, when comparing the idiopathic OAT group to the AS group, the OAT group displayed an even higher percentage of abnormal morphology and lower total and progressive motility % (Figures 1A, 1B, and Figure 2).

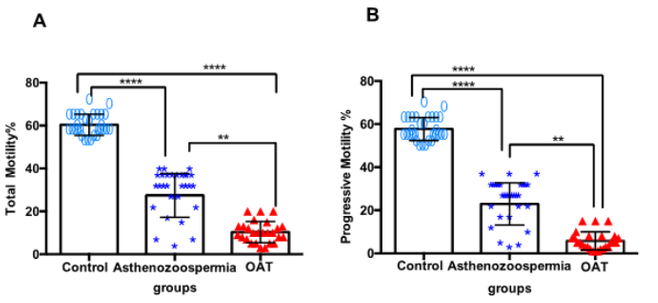

Figure 1. Presents the motility percentages of the OAT, asthenozoospermic, and control groups. Panel A illustrates the total motility, while Panel B displays the progressive motility data. Statistical analysis was performed using the, Kruskal-Wallis test. p<0.05* , p<0.01**, p<0.001***, p<0.0001****

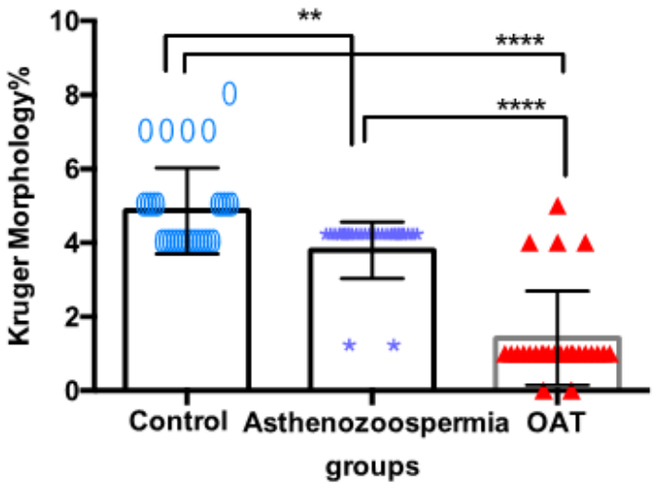

Figure 2. Comparison of Kruger morphology of levels in sperm cells of OAT, AS and control groups. Parameters were determined as described in the method. Bars represent median with interquartile range and each point represents a separate value. Group comparisons were performed using the Kruskal-Wallis test. p<0.05* , p<0.01**, p<0.001***, p<0.0001****

OAT: Oligoasthenoteratozoospermia, AS: Asthenozoospermia

Intracellular ATP levels and the ADP/ATP ratio were measured in sperm lysates from all samples. ATP levels were significantly higher in the OAT group compared with controls (p<0.05; Figure 3). No statistically significant differences in ATP levels were observed between the AS group and either the control or OAT groups. The ADP/ATP ratio was significantly higher in the OAT group compared with both the AS and control groups (p<0.0001; Figure 4).

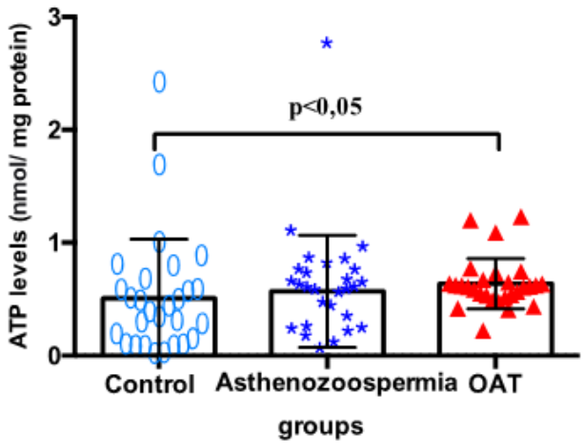

Figure 3. Comparison of ATP levels in sperm cells of OAT, AS and control groups. The parameter was determined as described in the method. Bars represent median with interquartile range and each point represents a separate value. Between groups was tested by the multi-sample comparison Kruskal-Wallis

ATP: Adenosine triphosphate, OAT: Oligoasthenoteratozoospermia,
AS: Asthenozoospermia

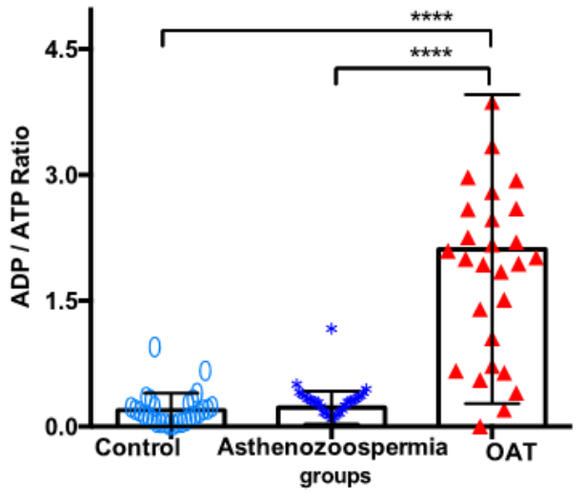

Figure 4. Comparison of ADP/ATP ratio in sperm cells of OAT, AS and control groups. The parameter was determined as described in the method. Bars represent median with interquartile range and each point represents a separate value. Between groups was tested by the multi-sample comparison Kruskal-Wallis. p<0.05* , p<0.01**, p<0.001***, p<0.0001****

ATP: Adenosine triphosphate, OAT: Oligoasthenoteratozoospermia,
AS: Asthenozoospermia

Table 1. Demographic characteristics and baseline of the study groups

Parameters	OAT (n=30)	AS (n=30)	Control (n=30)	p-value
Age (years)	31.70±5.91	32.50±6.12	33.17 ±5.86	p>0.05
BMI (kg/m²)	25.66±3.01	26.04±2.76	26.61±3.97	p>0.05
Abstinence (days)	3.90±0.95	4.16±1.17	3.90±0.95	p>0.05
Semen volume (mL)	2.33±1.01	2.91±1.15	3.00±0.83	p<0.05
Sperm concentration (×10⁶/mL)	9.14± 8.20	31.50±23.93	37.83±23.07	p<0.0001
Total motility (%)	10.38±4.94	27.47±10.25	60.37±4.96	p<0.0001
Progressive motility (%)	5.76±4.19	22.97±9.78	57.73±5.38	p<0.0001
Kruger morphology (%)	1.42±1.26	3.80±0.76	4.86±1.16	p<0.0001

Data are presented as mean ± SD
Statistical differences between groups were assessed using one-way ANOVA.
BMI: Body mass index, OAT: Oligoasthenoteratozoospermia, AS: Asthenozoospermia

Table 2. Spearman correlation values in OAT and AS groups

Parameters	Group r (Correlation coefficient)	p-value
Progressive motility % / Total motility %	OAT 0.96	p<0.001
	AS 0.99	p<0.0001
Kruger Morphology % ATP* levels	OAT 0.23	p>0.05
	AS 0.24	p>0.05
Progressive motility % / ATP levels	OAT 0.35	p>0.05
	AS 0.14	p>0.05
Progressive motility % / ADP/ATP ratio	OAT -0.84	p<0.001
	AS 0.04	p>0.05

ATP: Adenosine triphosphate, OAT: Oligoasthenoteratozoospermia, AS: Asthenozoospermia

Table 2 summarizes Spearman correlation values for various parameters in the OAT and AS groups. Values are presented as percentages, with correlation coefficients and p values in separate columns. In the OAT group, a strong negative correlation was observed between progressive motility and the ADP/ATP ratio (r=-0.84, p<0.0001). Additionally, a significant positive correlation was observed between progressive motility and total motility (r=0.96, p<0.0001). No significant correlations were found between ATP levels and Kruger morphology or progressive motility.

In the AS group, progressive motility was significantly correlated with total motility (r=0.99, p<0.0001), while no other parameters demonstrated significant correlations.

Discussion

In this study, we observed that OAT sperm exhibited higher ATP levels than AS sperm, despite reduced motility, suggesting that motility deficits may not be the primary determinant of sperm ATP content. Several studies have examined ATP levels in spermatozoa in relation to factors affecting their production (4,17,18). However, it remains unclear whether altered ATP levels are a cause or a consequence of sperm pathologies, creating a "chicken-and-egg" scenario. The present findings support the importance of evaluating AS and OAT as distinct pathological entities rather than as a single motility-impaired group. As emphasized in the introduction, grouping AS and OAT together may obscure critical differences in both energy metabolism and ATP utilization.

In our study, although both conditions exhibited reduced motility, OAT spermatozoa demonstrated a markedly higher ADP/ATP ratio and a strong inverse relationship with progressive motility, a pattern that was not observed in AS. These findings suggest that the metabolic consequences of motility impairment differ substantially between these pathologies, reinforcing the necessity of pathology-specific analyses when investigating sperm bioenergetics.

Both human and animal studies suggest that defects in metabolic pathways including mitochondrial oxidative phosphorylation, glycolysis, the Krebs cycle, fatty acid oxidation, and ketone body oxidation can impair sperm motility and contribute to male infertility (10,19). Lower motility generally correlates with reduced ATP, while higher ATP levels are often associated with better motility (14). Nevertheless, these relationships are complex, with multiple confounding factors affecting ATP dynamics. Given that spermatozoa primarily use ATP for motility, it is plausible that in cases where motility defects are not due to ATP production deficiencies, reduced ATP consumption could lead to ATP accumulation.

Measuring ATP consumption in individual sperm cells remains technically challenging. Although single-cell analysis methods are advancing, fully accounting for ATP-related mechanisms at this level is still difficult. Furthermore, previous studies often pooled distinct pathological sperm samples, potentially obscuring important differences. Comparing pathologies with similar motility impairments but differing underlying causes may provide insights into ATP accumulation and its relation to motility.

The ADP/ATP ratio is a widely recognized indicator of cellular energy status (20,21). Here, AS sperm showed a modest increase compared to controls, whereas OAT sperm exhibited a significantly higher ratio. Notably, a strong negative correlation was observed between the ADP/ATP ratio and progressive motility in OAT sperm. A high ADP/ATP ratio reflects a low-energy

state, implying either rapid ATP consumption or insufficient ATP utilization. The elevated ratio in OAT, despite higher ATP levels compared to AS and controls, likely represents an imbalance between ATP production and utilization, potentially compensating for accumulated unused ATP.

OAT presents a more complex phenotype than AS, including impaired motility, oligozoospermia, and teratozoospermia, all of which are ATP-dependent processes. Defective spermatogenesis further increases ATP demands. Although our study cannot pinpoint which specific ATP-consuming processes are impaired, it highlights the potential disruption of ATP production-utilization communication. Future research should investigate ATP compartmentalization and translocation mechanisms to better understand energy dysfunction in male infertility.

Conclusion

In conclusion, although ATP levels were higher in OAT sperm, the lack of a linear correlation with progressive motility suggests that motility deficits alone are unlikely to be the primary determinant of sperm ATP content.

Ethical Approval: *This study was approved by the Cerrahpaşa Faculty of Medicine Clinical Research Ethics Committee (approval no: A-51, date: February 2, 2021).*

Author Contributions:

Concept: İ.O., A.K.

Literature Review: M.A., A.K., N.G., İ.O.

Design: İ.O.,A.K.

Data acquisition: M.A., A.K., N.G., H.Ö.

Analysis and interpretation: İ.O., A.I, M.A.

Writing manuscript: İ.O., A.K.

Critical revision of manuscript: İ.0.,A.K.

Conflict of Interest: *The authors have no conflicts of interest to declare.*

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