Araştırma Makalesi (Research Article)

Semen pH ve Likefaksiyon Süresi Motilite ve Morfoloji Üzerinde Etkili midir?

Is Seminal pH and Liquefaction Time Effective on Motility and Morphology?

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Özet

Amaç: Likefaksiyon süresi ve semen pH değerlerinin sperm fonksiyonları ile ilişkili olup olmadığı konusunda yeterli çalışma bulunmamaktadır. Bu nedenle, bu çalışmada pH ve likefaksiyon süresi ile ana semen parametreleri arasında bir ilişki olup olmadığı değerlendirilmesi hedeflendi.

Gereç ve Yöntemler: İnfertilite açısından değerlendirilen 964 hastadan azospermi, şiddetli oligozoospermi (<1 milyon/ml) ve aynı katılımcının birden fazla semen örneği olanlar çıkarıldıktan sonra 600 hastanın semen örneği çalışmaya dahil edildi. Veriler normal ve anormal olarak gruplara ayrıldı ve pH ve likefaksiyon süreleri açısından karşılaştırıldı. Örnekler ayrıca semen pH değerine göre asidik (<7.2), normal (7.2-8.0) ve bazik (> 8.0) ve likefaksiyon süresine göre normal sıvılaştırma (≤30 dak.) ve yavaş sıvılaştırma (> 30 dak.) şeklinde gruplandırıldı, ve daha sonra konsantrasyon, motilite ve morfoloji bozuklukları açısından karşılaştırıldı.

Bulgular: 600 semen örneğinden 400'ü (% 66.7) anormal, 200'ü (% 33.3) normal olarak gruplandırıldı. Anormal grubun medyan pH ve sıvılaşma süresi normal gruptan istatistiksel olarak daha yüksekti (her ikisi de, p <0,01). Semen pH ve likefaksiyon süreleri astenoospermi, teratozoospermi ve astenoteratozoospermi gruplarında anlamlı olarak daha yüksekti (tümü için p <0.01). Bazik pH (> 8.0) grubunun konsantrasyonu, progresif motilitesi ve morfolojisi hem asidik hem de normal pH gruplarından anlamlı olarak daha düşüktü (hepsi için p <0.01). Bunun aksine, sıvılaşma zaman gruplarının morfolojileri arasında sadece anlamlı fark vardı (p = 0.02).

Sonuç: Anormal ve normal gruplarda semen pH ve likefaksiyon süresi değerleri anlamlı derecede farklıydı ve bu fark astenoospermi, teratozoospermi ve astenoteratozoospermisi olanlarda belirgindi.Bu ilişkileri daha iyi anlamak için kontrol grubu olarak kanıtlanmış fertil erkeklerle çalışmalar yapılmalıdır.

Anahtar Kelimeler: Likefaksiyon süresi, Morfoloji, Motilite, Semen pH

Abstract

Objectives: There are not enough reports about whether liquefaction time and semen pH values are related to sperm functions. Therefore, this study aimed to evaluate whether there is a relationship between pH and liquefaction time and major semen parameters.

Material and Methods: After excluding patients with azoospermia, severe oligozoospermia (<1 million/ml) and multiple semen samples from the same participant from 964 patients evaluated for infertility, semen samples of 600 patients were included in the study. The data were divided into groups as normal and abnormal and compared in terms of pH and liquefaction times. The samples were also grouped according to semen pH as acidic (<7.2), normal (7.2-8.0) and basic (>8.0) and liquefaction time as normal liquefying (≤30 min.) and slow liquefying (> 30 min.), and then were compared in terms of the normal/abnormal status of concentration, motility and morphology.

Results: Of the 600 semen samples, 400 (66.7%) were grouped as abnormal and 200 (33.3%) as normal. The median pH and liquefaction time of the abnormal group were statistically higher than the normal group (both, p <0,01). The seminal pH and liquefaction times were significantly higher in asthenozoospermia, teratozoospermia and asthenoteratozoospermia groups (for all, p <0.01). The concentration, progressive motility and morphology of the basic pH (>8.0) group was significantly lower than both acidic and normal pH groups (for all, p <0.01). Unlike, there was only significant difference between the morphologies of liquefaction time groups (p=0.02).

Conclusion: The seminal pH and liquefaction time values in the abnormal and normal groups were significantly different and this difference was evident in those with asthenozoospermia, teratozoospermia and asthenoteratozoospermia. To understand these relationships better, studies with proven fertile men as a control group should be performed.

Keywords: Liquefaction time, Morphology, Motility, Semen pH

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INTRODUCTION

Semen analysis is routinely performed during infertility assessment.The results of this procedure are guiding when making treatment decisions. Therefore, standartization of analysis is impotant. The World Health Organization (WHO) laboratory manual for the examination and processing of human semen was lastly revised in 2010, and lower reference limits for semen characteristics were standardized (1).The main parameters taken into attention in semen analysis are motility, morphology and concentration. Male factor infertility is considered, if abnormalities are detected in consecutive semen analysis according to WHO criteria.

Ejaculated semen is composed of spermatozoa and secretions of prostate and seminal vesicle(2).After ejaculation, first, a soft seminal coagulum is formed and then that liquefies macroscopically within 5-20 minutes at room temperature, generally completed within 30 minutes(3-5). Then, it is tried to discriminate liquefaction macroscopically and microscopically. Spermatozoa are immobile during liquefaction process and immobil spermatozoa can gain ability to move when semen liquefies.Semen analysis should not be started within 30 minutes in non-liquefied samples, and a further 30 minutes should be waited (1). In cases of infertility or subfertility, semen has been observed to have slow or non-liquefaction (6). Slow-liquefying samples that liquefied 30 minutes after ejaculation had more significant decrease in all the prostate-specific components of semen than normally liquefying ejaculates(5).

Semen pH is mainly determined by two accessory gland secretions , seminal vesicle and prostate, which are alkaline and acidic, respectively(1). The pH of seminal plasma may affect sperm function. The normal value of seminal fluid pH was accepted to be between 7.2 and 8.0 in WHO labarotory manual in 1992 revision. When the pH of the ejaculate is acidic (<7.2), there may be occlusion of the seminal vesicles, however, it may be related to infections with an alkaline pH (8.0)(7). Presently, there are not enough reference values for the seminal pH of fertile men. The last revised WHO laboratory manual did not change the consensus value of 7.2 as a lower threshold value. Therefore, new data is expected to be presented in the literature (1).

By the knowdledge that spermatozoa can gain movement by liquefaction, liquefaction time may affect semen parameters. There is not enough study about whether liquefaction time and semen pH values are related to sperm functions. While Banjoko et al. showed no significant difference in seminal pH values in hypomotile and normal motile groups (8), in another study patients with oligospermia or asthenospermia have been shown to have a semen pH <7.2(9). In the light of these data, we aimed to evaluate whether there is a relationship between seminal pH and liquefaction time and major semen parameters such as sperm motility, morphology and concentration.

MATERIAL and METHODS

Study Design and Samples

The semen analysis of patients attended or consulted for infertility in our Andrology Laboratory between July 2018 and March 2019 were reviewed retrospectively. The study was carried out appropriate to the latest version of the Declaration of Helsinki and approved by the Ethics Committee of Kahramanmaras Sutcu Imam University (approval number:118 - 2019/07).

In this laboratory analysis, 964 semen samples taken for infertility evaluation were obtained. After excluding samples with azoospermia and severe oligozoospermia (< 1 million/ml) and more than one samples of the same participants, 400 abnormal and 200 normal semen samples were included in the study.If all parameters of a semen sample such as semen volume, total sperm number, sperm concentration, total motility, progressive motility, vitality and sperm morphology were above the latest reference value recommended by the WHO, it was accepted as normal.To accept that the semen sample was abnormal, it was enough that only one of the parameters was below the reference value.

The data were divided into groups as normal and abnormal.The samples that also grouped according to status of oligozoospermia (O), asthenozoospermia (A), teratozoospermia (T), oligoasthenozoospermia (OA), oligoteratozoospermia (OT), astenoteratozoospermia (AT) and oligoasthenoteratozoospermia (OAT), were compared in terms of semen pH and liquefaction times. In addition, study samples were grouped according to semen pH as acidic (<7.2), normal (7.2-8.0) and basic (>8.0) and liquefaction time as normal liquefying (≤30 min.) and slow liquefying (> 30 min.), and then were compared in terms of the status of O, A, T, OA, OT, AT and OAT.

Semen analysis

After a sexual break of 3-5 days, semen samples were collected in the laboratory by masturbation and the analysis was performed according to the latest WHO criteria. After the specimens were incubated at 37 ° C for liquefaction, the liquefaction times were recorded. Then, the volume and pH of the samples were measured by gravimetric and volumetric methods and by pH strip, respectively. The analysis was performed under a phase-contrast microscope (Olympus). Samples were analyzed for sperm motility, vitality, concentration and morphology.

Statistical Analysis

The median (interquartile range (IQR)) and the percentage of data from the basic descriptive statistics were used for all study groups. Due to the abnormal distribution of data, Mann-Whitney U test and Kruskal Wallis test were used for comparison of two groups and more than two groups, respectively.Chi-Square test was used for testing relationships between categorical variables.The statistical significance was defined as $p<0.05$ and the results evaluated at 95% confiden-

ce interval. For all statistical analysis, SPSS software (version 22.0, IBM, USA) was used.

RESULTS

The median (IQR) age, pH and liquefaction time of all data were 31 (28-34), 7.5 (7.0-8.0) and 20 (20-30), respectively. Of the 600 semen analysis results, 400 (66.7%) were grouped as abnormal and 200 (33.3%) as normal. In all cohort, the groups with the highest percentages were A (64.3%), T(62.8%), AT (60.5%), normal pH(7.2-8.0) (40.7%) and normal liquifying (≤30 min) (82.5%) groups **(Figure 1).** In the group with the abnormal semen analysis, 278 (69.5%) patients had a semen pH ≥ 7.2, whereas in the normal group, the pH of 112 (56%) patients was <7.2.

The median pH and liquefaction time of the group with abnormal semen analysis were statistically higher than the normal group(both, p <0,01) **(Table 1).** The seminal pH and liquefaction times were significantly higher in A, T and AT groups (p <0.01 for all). However, there were no significant differences in pH and liquefaction time of O, OA, OT and OAT groups **(Table 2).**

There were significant differences between concentrations, progressive motilities and morphologies of semen pH groups (for all, p <0.01). The concentration, progressive motility and morphology of the basic ph group was significantly lower than both acidic and normal ph groups (for all, p <0.01). There was also significant difference between the morphologies of acidic and normal ph groups (p <0.01).

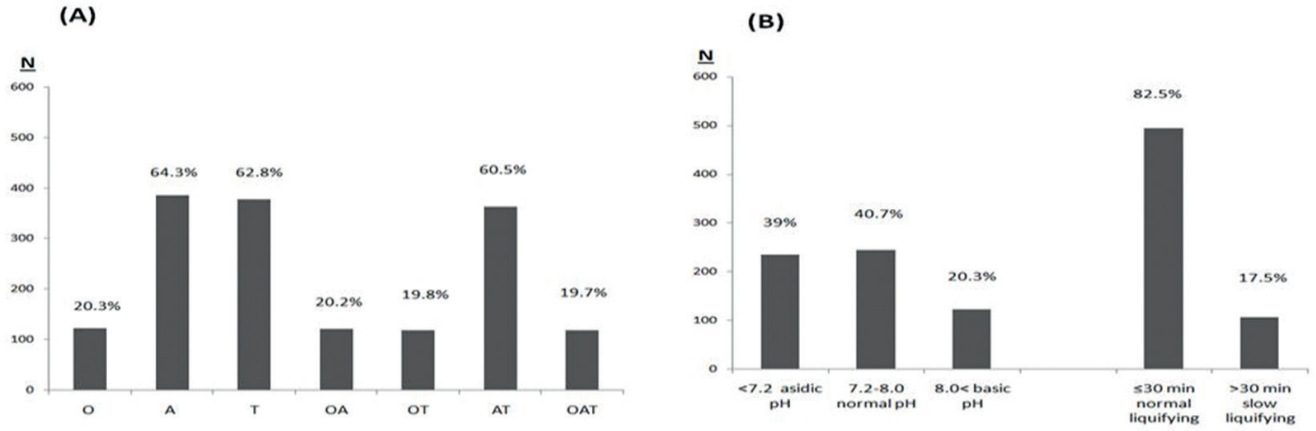


Figure 1. The persentage and count of semen parameters (A). The persentage and count of O,A,T,OA,AT and OAT groups, (B). The persentage and count of seminal pH and liqefaction time groups.

Table 1. Comparison of demographics and sperm parameters between abnormal and normal semen analysis groups.

	Abnormal semen analysis (N:400)	Normal semen analysis (N:200)	p* value
Age	32 (28-36)	31 (29-35)	0.56
Volume (ml)	3.4 (2.6-4.6)	3.2 (2.6-4.4)	0.83
pH	8.0 (7.0-9.0)	7.0 (7.0-8.0)	***<0.01***
Liquefaction time (min)	25 (20-30)	20 (20-25)	***<0.01***
Concentration (million/ml)	24 (12-42)	53 (32-68)	***<0.01***
Progressive motility (%)	18 (10-24)	36 (32-38)	***<0.01***
Morphology (%)	0 (0-2)	4 (4-6)	***<0.01***
Vitality (%)	65 (59-70)	74 (73-76)	***<0.01***

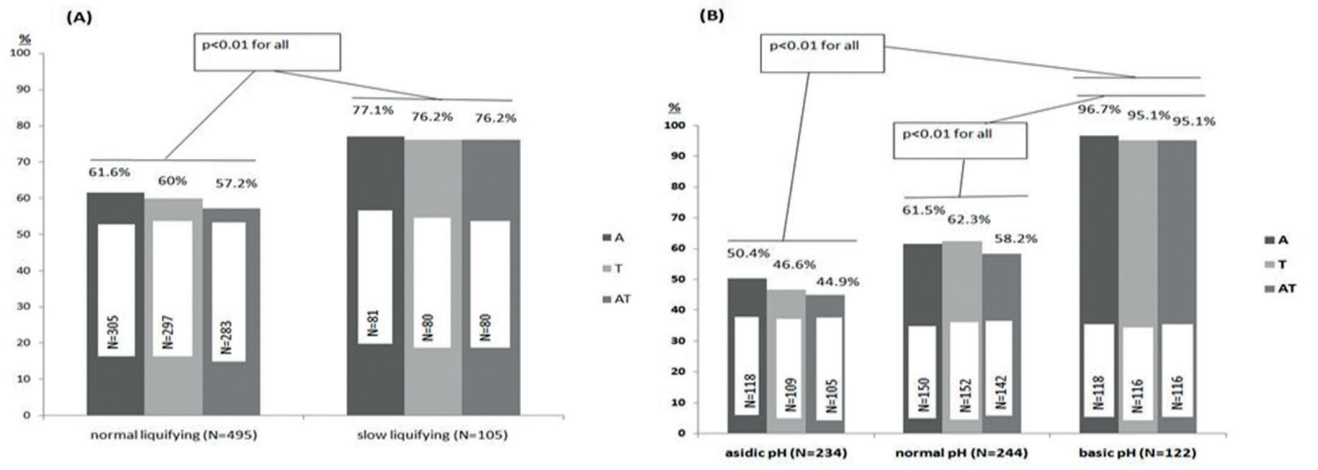
*Comparison between groups were made using Mann-Whitney U test. Summary statistics are presented as median (interquartile range). Statistical significance was at p<0.05 and shown in bold and italic.

Unlike, it was observed that there was only significant difference between the morphologies of liquefaction time groups (p=0.02) **(Table 3).** In addition, O, A, T, OA, OT, AT and OAT rates were examined in ph and liquefaction subgroups. There were differences in only A, T and AT rates of both ph and liquefaction subgroups. Semen samples with slow liquefying had higher rates of A, T and AT than normal liquefying ones (for all p<0.01). Semen samples with basic pH had higher rates of A, T and AT than both acidic and normal pH ones (for all p<0.01) **(Figure 2).**

Table 2. Seminal pH and liquefaction time difference between O, A, T, OA, OT, AT and OAT status groups.

		pH Median (IQR)	p value	**Liquefaction time** Median (IQR)	p* value
O status	*Yes*	7.5 (7.0-9.0)	0.20	20 (20-30)	0.97
	No	7.5 (7.0-8.0)		20 (20-30)	
A status	*Yes*	8.0 (7.0-9.0)	**<0.01**	25 (20-30)	***<0.01***
	No	7.0 (7.0-8.0)		20 (20-25)	
T status	*Yes*	8.0 (7.0-9.0)	**<0.01**	25 (20-30)	***<0.01***
	No	7.0 (7.0-8.0)		20 (20-25)	
OAT status	*Yes*	7.5 (7.0-9.0)	0.16	20 (20-30)	0.91
	No	7.5 (7.0-8.0)		20 (20-30)	
OA status	*Yes*	7.5 (7.0-9.0)	0.16	20 (20-30)	0.99
	No	7.5 (7.0-8.0)		20 (20-30)	
OT status	*Yes*	7.5 (7.0-9.0)	0.19	20 (20-30)	0.87
	No	7.5 (7.0-8.0		20 (20-30)	
AT status	*Yes*	8.0 (7.0-9.0)	***<0.01***	25 (20-30)	***<0.01***
	No	7.0 (7.0-8.0)		20 (20-25)	

*Comparison between groups were made using Mann-Whitney U test. Summary statistics are presented as median (IQR). Statistical significance was at p<0.05 and shown in bold and italic. O: Oligozospermia, A: Asthenozospermia, T: Teratozoospermia, OA: Oligoasthenozoospermia, OT: Oligoteratozoospermia, AT: Asthenoteratozoospermia and OAT: Oligoasthenoteratozoospermia



Note. Chi-square test was used for statistics. Bonferroni correction was used for p value in B. Statistical significance was at p <0.05. A: Asthenozospermia, T: Teratozoospermia and AT: Asthenoteratozoospermia

Figure 2. Differences of A,T and AT in semen pH and liquefaction groups.

Table 3. Comparison of concentration, progressive motility and morphology in seminal pH and liquefaction time subgroups.

		Concentration (million/ml)		Progressive motility(%)		Morphology (%)	
		Median (IQR)	p	Median (IQR)	p	Median (IQR)	**p**
*pH groups	Asidic pH	33.5 (21.0-56.0)		31.0 (17.8-36.0)		4.0 (0.0-5.0)	
	Normal Ph	32.0 (19.0-58.0)	***<0.01*[a]**	24.0 (14.3-32.0)	***<0.01*[b]**	2.0 (0.0-4.0)	***<0.01*[c]**
	Basic pH	27.0 (13.0-40.5)		19.0 (8.0-22.0)		0.0 (0.0-2.0)	
**Liquefaction time groups	Normal(≤30min)	32.0 (17.0-54.0)	0.85	24.0 (13.0-32.0)	0.26	2.0 (0.0-4.0)	***0.02***
	Slow (>30min)	32.0 (21.5-46.0)		22.0 (14.0-28.5)		1.0 (0.0-3.0)	

Note. Summary statistics are presented as median (IQR). Statistical significance was at p<0.05 and shown in bold and italic.
*. Comparison between groups was made using Kruskal-Wallis analysis and post hoc multiple comparisons were made Mann-Whitney U test with Tamhane's correction.
**. Mann-Whitney U test was used for comparison.
[a].p <0.01 for ph asidic pH vs basic pH groups and normal pH vs basic pH groups (post hoc analysis).
[b].p <0.01 for ph asidic pH vs basic pH groups and normal pH vs basic pH groups (post hoc analysis).
[c].p <0.01 for all group comparisons (post hoc analysis).

DISCUSSION

The normal value of seminal fluid pH was accepted to be between 7.2 and 8.0 in WHO labarotory manual in 1992 (7), than the lower threshold value of 7.2 was accepted as the consensus value in last version of WHO labarotory manual (1). Currently, there is not enough reference value for the pH of semen from fertile males.Therefore, the last manual pends more data for revision.

In the semen analysis of 207 men who underwent infertility evaluation, the seminal ph was consistently above 8.0 by using ph meter and ph paper at different times (30 and 60 min)(10). Harraway et al. with median 8.2 (range 7.3-9.5), semen pH values of the populations (N = 1199) were higher than WHO reference values (7.2-8.0). İn addition, the semen pH values of 602 patients with normal sperm concentration and motility and 597 patients with abnormal parameters were similar (both medians, 8.2) (11).In our study, the median pH of the abnormal semen analysis group was 8.0 (IQR, 7.0-9.0) and the number of patients with pH ≥ 7.2 was 278 (69.5%). Moreover, the normal semen analysis group which was normozoospermic had 112 (56%) samples with pH <7.2. As shown in these reports and our study, pH could be higher than 7.2 in populations with abnormal semen parameters and could be lower than 7.2 in the normozoospermic patients (for semen ph ≥ 7.2 for fertile men in last WHO). Although the normal semen analysis group in our study did not fully represent samples from normal fertile men, our results also supported the need for new studies on pH value.

In a study of 80 semen samples taken from men admitted to the infertility clinic, patients were divided into groups as hypomotility and normal motility according to 60% motility and no difference was found between the ph values of the groups (7.51±0.02 and 7.54±0.02, respectively), (p=0.21)(8). In our study, the pH values in the abnormal and normal semen analysis groups were statistically different (median 8.0 (IQR,7.0-9.0) and 7.0 (IQR,7.0-8.0), respectively, p<0.01)). In the subgroup analysis, this difference was found to be related to motility and morphology and ph values were higher in the groups with A and T (p<0.01).

In another study of 136 fertile healthy men, the purified sperm were resuspended in the sperm feeding solution of pH 5.2, 6.2, 7.2, and 8.2 and divided into groups. It was revealed that the pH 7.2 and 8.2 appeared to be the optimal condition for total motility and progressive motility of human sperm (9).At the present study, semen samples with basic pH (>8.0) had higher rates of A, T and AT than both acidic (<7.2) and normal pH (7.2-8.0) ones (for all p<0.01). This difference may be due to more alkaline pH (>8.0) usually associated with infections(7) that may affect motility and morphology.

Ejaculated semen is composed of spermatozoa and prostate and seminal vesicle secretions.After the semen coagulum forms, it begins to liquefy and is usually completed within 30 minutes (3-5). In the literature, there was no study comparing liquefaction time with main semen parameters such as concentration, motility and morphology. In our report, the liquefaction time was longer in the abnormal sperm analysis group, especially in the A, T and AT subgroups than in normozoospermic patients (p<0.01). In addition, only the morphology of the slow liquifying group was lower than the normal liquefying group (p=0.02). Furthermore, the slow liquefying group had higher rates of A, T and AT than the normal

The present study has a few limitations, including its retrospective nature, relatively few samples and/or the absence of a proven fertile control group. Nevertheless, this study provides valuable information about the relationship of ph and liquefaction time to the main semen parameters.

As a conclusion;The seminal pH and liquefaction time values in the abnormal and normal semen analysis groups were significantly different and this difference was evident in

those with A, T and AT.While all three main parameters were significantly affected in basic ph (>8.0), only morphology was significantly affected in those with slow liquefying (>30 min) samples. However, both the basic pH and slow liquefying samples had higher rates of A, T and AT. To understand these relationships better, studies with proven fertile men as a control group should be performed.

Conflict of Interest and Financial Status

Conflict of Interest and Financial Status:Our study has not been financed by an institution and institution. In this study, there is no conflict of interest among the authors on any subject.

Author Contribution

All authors contributed equally to the article.

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