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Rheology of Human Seminal Fluid

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SUMMARY. The clinical manifestation known as asthenozoospermia has a multifactor origin and it reveals itself as the damage to one of the major functional characteristics of human spermatozoon: the rapid linear progressive motility (grade a).In many cases of asthenozoospermia the prevalent biophysical alteration is the high viscosity of human seminal fluid. The correlation between seminal consistency and viscosity of human seminal fluid was studied. Viscosity was determined using a Wells-Brookfield (rotational) and a modified Ostwald (capillary) viscosimeter and consistency was established by introducing a glass rod into the sample and observing the length of the thread that forms on withdrawal of the rod (normal < 2 cm). The rheological variables considered: apparent viscosity at maximum shear rate (η_{am}), yield value (τ_o), thixotropy area (μ) (rotational viscosimeter), and relative viscosity (η_r) (capillary viscosimeter) presented statistically significant differences when patients were grouped according to the consistency of their seminal fluid ($p < 0.05$ for the η_{am} and $p < 0.0001$ for the remaining variables). Working at room temperature (20 ± 2°C) with seminal fluids of normal consistency (n = 67) ($\bar{x}$ ± 2 SEM) η_{am} was 4.3 ± 0.4 cp; τ_o was 0.32 ± 0.02 dynes/cm^2 and μ was 47.1 ± 7.6 dynes/cm^2.sec, while with highly viscous seminal fluids (n = 19) the values were: 5.4 ± 0.8 cp, 0.83 ± 0.16 dynes/cm^2 and 218.2 ± 72.4 dynes/cm^2.sec, respectively. The η_r at room temperature of normal consistency seminal fluids (n =117) was 5.1 ± 0.4 and for highly viscous semen (n = 44) the values obtained were 15.2 ± 4.0. Working at room temperature (20 ± 2 °C) and at 37 °C, the η_{am} and μ showed no statistically significant difference between normal and high viscosity groups, while in the other considered rheological variable, τ_o, the significant difference ($p < 0.0001$) could be due to the behaviour of the material at low shear rates. Determinations performed within 2, 3 and 4 hours after semen emission showed no differences, assuming that the four hour span after ejaculation has no incidence on the η_r. In the determination of η_r no diluent can be used, since the observed decrease in the viscosity was not proportional to the dilution performed. The methods used (rotational and capillary) are not comparable due to the different geometry employed.

RESUMEN. "Reología del semen humano". El cuadro denominado astenozoospermia posee origen multifactorial y se visualiza como el daño a una de las características funcionales más importantes del espermatozoide humano, la movilidad progresiva lineal rápida (grado a), siendo la consistencia seminal aumentada la condición biofísica de mayor compromiso. En el presente trabajo se estudió la correlación de la consistencia seminal con la viscosidad del semen humano, determinada con los viscosímetros rotacionales Wells-Brookfield y de Ostwald modificado. Los parámetros reológicos considerados: viscosidad aparente a la máxima velocidad de corte (η_{am}), valor de cedencia (τ_o) y área de tixotropía (μ) (viscosímetro rotacional) y viscosidad relativa (η_r) (viscosímetro capilar), presentaron diferencias matemáticamente significativas al ser agrupados de acuerdo a su consistencia ($p < 0.05$ para el primero y $p < 0.0001$ para los restantes parámetros). Trabajando a 20 ± 2 °C , con sémenes de consistencia normal (n = 67), la η_{am} ($\bar{x}$ ± 2 ES) fue de 4.3 ± 0.4 cp; el τ_o fue de 0.32 ± 0.02 dinas/cm^2 y μ fue de 47.1 ± 7.6 dinas/cm^2 seg, siendo de 5.4 ± 0.8 cp, 0.83 ± 0.16 dinas/cm^2 and 218.2 ± 72.4 dinas/cm^2 seg, respectivamente, en sémenes de consistencia aumentada (n = 19). La η_r en sémenes de consistencia normal (n = 117) resultó 5.1 ± 0.4 y con consistencia aumentada (n = 44) los valores obtenidos fueron de 15.2 ± 4.0. Trabajando a 20 ± 2 °C y a 37 °C, η_{am} y (no mostraron diferencia estadísticamente significativa, mientras que en el otro parámetro reológico considerado, τ_o, la significativa diferencia ($p < 0.0001$) podría deberse al comportamiento del material a bajas velocidades de corte. El lapso de hasta 4 h de producida la eyacuación no incide sobre la η_r, observándose que determinaciones realizadas a 2, 3 y 4 h no muestran diferencias. En la determinación de la η_r no puede recurrirse al uso de diluyentes, por cuanto la caída de la viscosidad observada no es proporcional a la dilución realizada. Los métodos utilizados no son comparables, hecho adjudicable a la distinta geometría empleada.

KEY WORDS: Asthenozoospermia, Rheology, Semen
PALABRAS CLAVE: Astenozoospermia, Reología, Semen.

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INTRODUCTION

Alterations of the rapid linear progressive motility of spermatozoa, asthenozoospermias, constitute a pathology of significant incidence in male infertility. Asthenozoospermias are a result of morphological and/or functional alterations of the spermatozoa [1,2], but there are also cases in which the cause is high viscosity of seminal fluid [3].

Due to the importance of the seminal fluid behaviour, several rheological studies have been done using viscosimeters of different geometry, *i.e.* capillary [4-6] and rotational [7] ones.

As a consequence of ejaculation there is a mixing of the secretions from the principal annex glands and coagulation of the sperm takes place, followed by changes which lead to liquefaction. This coagulation-liquefaction process has an enzymatic origin [8]. The viscosity of seminal fluid is a different and independent phenomenon from the coagulation-liquefaction process [9]. In some seminal fluids high viscosity is observed after the coagulation-liquefaction process has taken place, while in others it occurs with total or partial lack of liquefaction. The cause of high residual viscosity is only partially known [10].

According to the WHO standarization [11], when studying human seminal fluid, viscosity is estimated in terms of seminal consistency. The aim of this work has been to study the viscosity of human seminal fluid determined using rotational and capillary viscosimeters, its correlation with seminal consistency and the effect of temperature, time and dilution on viscosity, in normal and abnormal subjects.

METHODS

The collection and analysis of semen was carried out according to the procedures recommended by the World Health Organization (WHO). All samples were macroscopically liquefied at the time of measurement. The semens were grouped based on whether their consistency was normal or high. The consistency (often referred to as "viscosity") of the liquefied semen can be estimated by introducing a glass rod into the sample and observing the length of the thread that forms on withdrawal of the rod; the thread should not exceed 2 cm in normal samples. Since the measurement of semen consistency is performed at room temperature, this same temperature was used to determine its viscosity, so as to obtain comparable data.

Viscosity measuring methods

a) *Apparent viscosity.* A Wells-Brookfield rotational viscosimeter, which allows for eight shear rates, was used [12]. The readings were done every three minutes starting from: 1.15sec^{-1} to 230 sec^{-1}; therefrom, the reverse procedure was followed. The corresponding rheograms were plotted (shear stress *vs.* shear rate) and from these graphs both the apparent viscosity at maximum shear rate (η_{am}, cp) as well as two other rheological variables were obtained: yield value (τ_o, dyne/cm^2) and thixotropy area μ, dyne/cm^2.sec, determined by the sum of trapezoids method). The rotational viscosimeter used requires 1.0 ml of semen and can be used at room temperature (20 ± 2 °C) and at 37 °C. This last temperature was used in 21 samples to prove the existence of changes, if any, when compared to room temperature, used routinary in this work.

b) *Relative viscosity.* Capillary viscosimeters were used designed on the basis of the Ostwald viscosimeter, adapted for 2.5 ml volumes.

To assess the effect of semen dilution on the relative viscosity (η_r), the determinations were done on undiluted samples and on 1:2 and 1:4 dilutions with phosphate buffered saline (PBS, NaCl 7.75 g/l; K_2HPO_4 1.50g/l; KH_2PO_4 0.20g/l, pH: 7.6).

The time span effect on η_r was studied by performing measurements at 120, 180 and 240 min after ejaculation.

Statistical Analysis

The mean and standard error (SEM) was calculated for all the variables studied. Fisher's test was applied to compare variances. On comparing two populations Student's test was used if variances showed no significant difference according to Fisher's test; if not, the Welche's test was employed instead. Data were subjected to analysis of variance when more than two groups were compared. The regression analysis was used to determine the existing correlation in cases of two variables under study.

RESULTS

The validity of grouping the material for rheological studies according to its consistency was assessed using rotational and capillary viscosimeters. Working with a rotational viscosimeter the results demonstrate that in the 67 cases with normal consistency the η_{am} ($\overline{X}$ ± 2 SEM) was 4.3 ± 0.4 cp, while in 19 samples with high consistency the value was 5.4 ± 0.8 cp ($p < 0.05$, Table 1). Moreover, in 117 cases of normal consistency the η_r value was 5.1 ± 0.4 while in 44 ejaculates with high consistency the value was 15.2 ± 4.0, establishing mathematically that when determining the η_r (Table 2) there is a similar finding though mathematically more relevant than when working with the η_{am}, since, once again, two different populations are defined ($p < 0.0001$).

The determination of rheological variables (rotational viscosimeter) at laboratory temperature (20 ± 2 °C) and 37 °C demonstrates that η_{am} as well as μ show no difference in the values obtained at the mentioned temperatures ($p > 0.05$), while the yield value (τ_o) gives a statistically significant difference between both groups ($p < 0.0001$, Table 3).

The determination of the η_r (Ostwald method) at 2, 3, and 4 hours after ejaculation shows that there are no statistically significant differences with regard to the effect of time (Table 4).

Rheological variables	Normal consistency (n:67)		High consistency (n:19)
η_{am} (cp)	4.3 ± 0.4	p<0.05	5.4 ± 0.8
τ_o (dina/cm^2)	0.32 ± 0.02	p<0.0001	0.83 ± 0.16
μ (dina/cm^2.sec)	47.1 ± 7.6	p<0.0002	218.2 ± 72.4

Table 1. Seminal consistency and rheological variables determined with a rotational viscosimeter at room temperature. Data expressed as x ± 2 SEM.

	Normal consistency (n: 117)		High consistency (n: 44)
Viscosity (relative)	5.1 ± 0.4	p<0.0001	15.2 ± 4.0

Table 2. Seminal consistency and relative viscosity determined with a capillary viscosimeter at room temperature. Data expressed as x ± 2 SEM.

Rheological variables	Room temperature (n:86)		Temperature 37 °C (n:21)
η_{am} (cp)	4.5 ± 0.4	NS	4.5 ± 0.6
τ_o (dina/cm^2)	0.43± 0.04	p<0.0001	0.26 ± 0.08
μ (dina/cm^2.sec)	84.9 ± 16.8	NS	78.9 ± 36.8

Table 3. Rheological variables determined with a rotational viscosimeter at room temperature and at 37 °C. Data expressed as x ± 2 SEM.

Time (hours)	Viscosity (relative) (n:13)	
2	7.4 ± 3.8	
		NS
3	6.9 ± 3.2	
		NS
4	6.3 ± 2.8	

Table 4. Effect of the time span on the relative viscosity. Data expressed as x ± 2 SEM. Analysis of variance: NS.

n: 29	Viscosity (relative)	
samples undiluted	14.4 ± 5-8	
		p<0.002
1:2 dilution	6.2 ± 1.0	
		p<0.002
1:4 dilution	6.8 ± 0.8	

Tabla 5. Effect of semen dilution on the relative viscosity with phosphate buffer saline. Data expressed as x ± 2 SEM.

The correlation observed in 29 samples between the η_r determination (Ostwald method) performed in undiluted samples and in the same samples diluted 1:2 and 1:4 with PBS gave r: 0.4728 and r: 0.4487 for the first and second dilution, respectively (Table 5).

The use of two different methods established the need to study if there exists a linear correlation between the values obtained for η_r (capillary method) and η_{am} (rotational viscosimeter). The results obtained from 20 samples using the regression analysis give r: 0.0251.

DISCUSSION

The measurement of viscosity of human seminal fluid presented a drawback when selecting the viscosimeters to be used which are limited to those requiring reduced volumes, because, due to the volume ejaculated and the need to do other determinations, the mean volume available oscilates between 1.5 and 2.0 ml. This

requirement is fulfilled by the rotational viscosimeters and by the capillary geometry viscosimeters designed on the basis of the Ostwald viscosimeter.

The determination of seminal consistency performed in standard conditions is a good estimate of seminal viscosity, allowing for a classification of the semen in two groups: normal and high consistency, which correlate mathematically with the viscosity of the material, since in 86 samples, 67 with normal consistency and 19 with high consistency, the rheological considered variables showed a statistically significant difference ($p < 0.05$) for η_{am} and a relevant significant difference for τ_o ($p < 0.0001$) and μ ($p < 0.0002$) and using capillary viscosimeters (Ostwald method) the existing correlation between the η_r and seminal consistency, define two different groups ($p< 0.0001$). It is worth noticing that in those patients with increased consistency, whose values were below 5.5 (n:4) it should be taken into account that this fact could be due to the handling of the material during the determination of viscosity (mechanical stress applied to the material due to multiple passages through a capillary tube), while in those with remarkably high values (n:3) it must be considered that they could be due to technical artifacts produced by small clots unseen macroscopically, retarding the fluid flow in the capillary lumen.

The determination of seminal consistency is performed at room temperature; thus, we consider that this same temperature must be used to assess the viscosity, even if the obtained results show that there is no statistically significant difference in the η_{am} determined at laboratory temperature and at 37 °C; the same happens with μ, while the significant difference seen in the other rheological considered variable, τ_o, could be due to structural changes of the material particularly at low shear rates.

The 4 hour span after ejaculation has no effect on the ηr; measurements performed after 2, 3, and 4 hours show no statistically significant difference ($p > 0.05$).

The factor affecting η_r in the dilution of the semen is the addition of the diluent and not the volume added, thus allowing to suppose that the diluent causes physical changes apart from chemical modifications; therefore, in the determination of η_r we could not use the diluent to solve the quantification of viscosity when the amount of semen available is below that those required by the viscosimeters in use.

One of the methods used (Ostwald viscosimeter), gives the ηr values of the samples while the other, (rotational viscosimeter), gives the η_{am} ; both determinations correlate with the seminal consistency, but the data obtained confirm the hypotesis that both methods do not compare with each other due to the different geometry employed.

Capillary viscosimeters are cheap and simple to operate. Rotational viscosimeters have the advantage of working at different shear rates. In agreement with other authors [7], we observed that rotational viscosimeters do not have the problem of plugging the flow, which in some cases can be seen with capillary viscosimeters, but their drawback is that the material is evidently modified during the determination.

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