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Effect of type and severity of semen viscosity on sperm parameter, antisperm antibody, some cytokine and zinc levels for infertility men

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***Abstract*---**viscosity of semen is one of the important factors in the occurrence of fertilization. Increasing the viscosity leads to infertility because it affects the function of the sperm, it linked to a decrease in the movement of sperm, and thus prevents the passage of sperm through the female reproductive system naturally. The aim of this study was to assess the effect type (normal or abnormal) and severity of semen viscosity (mild, moderate, sever) on semen quality, antisperm antibody, some cytokines and zinc levels for infertile men. The study included of eighty-eight (88) semen samples from individuals that are in abstinence for 2-7 days where the study included 34 men had normal semen viscosity and 54 men had abnormal semen viscosity from Fertility Centre in Al-Sadder teaching hospital in Al-Najaf / Iraq during the period of (2/11/ 2021) and (5/2/2022). The results of current study showed a nonsignificant decrease at ($p<0.05$) in sperm parameters. while showed a nonsignificant increase at ($p<0.05$) in leucocytes in group abnormal semen viscosity as compared with group normal semen viscosity also in sever semen viscosity compared to moderate semen viscosity. Also the result showed nonsignificant increase ($P\leq0.05$) in antisperm antibody, interluekine 6 and nonsignificant decrease at ($p<0.05$) in interluekine1and zinc levels in abnormal viscosity compared to normal semen viscosity while they revealed elevation in sever semen viscosity compared to moderate and mild semen viscosity in antisperm antibody, Interleukin 1B, Interleukin 6 and dropped in zinc levels our results showed that semen viscosity (abnormal viscosity) and severity has a negative effect on semen quality and levels of antisperm antibody (ASA), cytokines and zinc (increase in ASA, interleukin 1, 6 and decrease in zinc) Therefore, the semen viscosity is one of causes of male infertility and prevent the occurrence of pregnancy.

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Introduction

Infertility is a worldwide problem that is defined as the failure of a couple to conceive after one year of regular unprotected intercourse, affecting 10 to 15 percent of couples, according to the most recent WHO estimates. Between 50 and 80 million people worldwide suffer from infertility (Babakhanzadeh, et al.,2020) In 70% of cases, there are identifiable causes for male infertility, such as anatomical, immunological, physical or obstructive diseases, hormonal and environmental variables. However, in 30% of cases, there is no clear explanation for male infertility, which is referred to as idiopathic factors (A Rivera-Diaz, et al.,2021). Semen is comprised of fluids released by the male accessory glands, which contain proteins necessary for its coagulation and liquification. The abnormal viscosity of seminal fluid caused by dysfunction of the prostate or seminal vesicles (Mahran, Z., and Saleh, 2014) is characterized by a thick and coagulated appearance (Issa Layali, et al.,2015). It affects approximately 12 to 29 percent of male infertile individuals (Du Plessis et al., 2013). A rise in seminal fluid viscosity can cause severe harm to the fluid's components and qualities (Elia, etal, 2009)

High viscosity can interfere with the evaluation of sperm motility, sperm concentration, antibody-coated spermatozoa, and biochemical indicators (WHO.,2010). Male accessory gland infection/inflammation (MAGI) consists of infection and inflammation of the epididymis, prostate, and/or seminal vesicles. Seminal fluid hyperviscosity is frequently associated with MAGE (La Vignera, etal.,2014). Leukocytes can potentially play a significant role in hyperviscosity development (Harchegani, etal.,2019). Increased seminal leukocyte counts lead to oxidative stress (OS) (Du Plessis et al., 2013). Recent research demonstrates that OS generated by ROS is the primary mechanism of HSV on poor sperm quality and male infertility in hyperviscous semen patients (Issa Layali, et al.,2015)

Semen hyperviscosity can be successfully treated in vitro using a 5-mL syringe with a sterile 18G needle. The specimen was pulled gently into the syringe and slowly ejected back into the tube (Esfandiari, etal,2008). - chymotrypsin was also effective in the treatment of SHV in an in vitro setting, such as IVF or IUI (Honea, et al,1993). Patients with less severe SHV can be treated in vivo with anti-inflammatory drugs, however patients with severe SHV responded positively to treatment (Elia, etal,2009). SHV infections and leukocytospermia may potentially be treated with antibiotics in vivo (Munuce, 1999).

Aim of the work

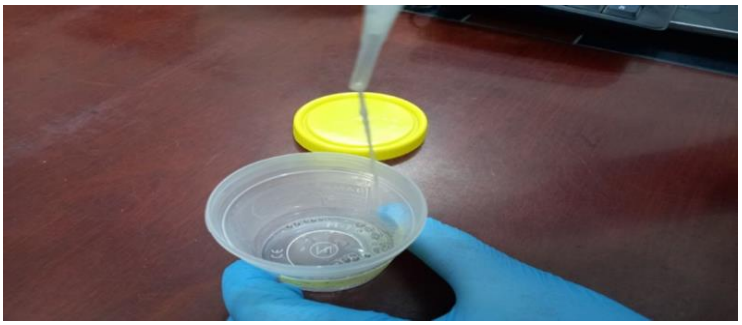
Study effect type of semen viscosity (normal or abnormal) and severity of semen viscosity on semen quality, antisperm antibody, some cytokines and zinc levels for infertile men

Participants and Methods

The present study was conducted in the infertility center of Al-Sader Medical city in Al-Najaf governorate and in the laboratory of advanced research of the Department of Laboratory Investigations/ Faculty of Science/ University of Kufa. where carried out between (2/11/ 2021) and (5/2/ 2022) the study included a total of eighty-eight (88) semen samples where patients are divided to normal semen viscosity (34) and abnormal semen viscosity (54) of these (38) infertile men with sever viscosity and 16 with moderate and mild viscosity. Semen parameters and levels of antisperm antibody, some cytokine and zinc were studied for the normal semen viscosity and compared to the abnormal semen viscosity, also between sever semen viscosity compared to moderate and mild semen viscosity

Laboratory evaluation

- Semen collection and evaluation
  Semen samples were collected from patients after 2–7 days of abstinence,
  The semen samples were examined after they were left in the incubator at 37ºC to liquefy completely for about 30-60 minutes. After completing the liquefaction and removed abnormal viscosity the parameters of semen and sperm were examined microscopically and macroscopically. These parameters include: sperm concentration, sperm motility percent, progressive motility, sperm morphology percent and leukocytes, then the sample was centrifuged at 3000 (rpm) for 10 minutes to obtain the plasma. The seminal plasma was frozen in –20 Co for examination of antisperm antibodies, cytokine and zinc levels.
  The male partners were examined and the reproductive aspect is evaluated by the semen analysis according to the world health organization WHO (2010)
- Semen viscosity evaluation
  the viscosity of the sample can be estimated by gently aspirating it into a wide-bore (approximately 1.5 mm diameter) plastic disposable pipette, allowing the semen to drop by gravity and observing the length of any thread. A normal sample leaves the pipette in small discrete drops. If viscosity is abnormal, the drop will form a thread more than 2 cm long (Figure 1)



hyperviscosity was graded under normal gravity as being mild (length of thread,>2 and ≤4 cm) moderate (>4 and ≤6 cm), or severe (>6 cm).

- Semen Leukocytes evaluation
  Leukocytes were counted using the Leucoscreen method,Briefly, one drop of semen was mixed with one drop of working solution (Leucoscreen stain and hydrogen peroxide), covered with a cover slip for 2 min, and then the result was read at a magnification of ×400. Peroxidase-positive cells stained yellow to brown, whereas other cells stained pink (Mahran, and Saleh., 2014).

Calculate the proportion of peroxidase-positive cells as follows

Proportion positive round cells

$$\frac{\text{Number of positive round cells}}{(\text{Number of positive round cells} + \text{Number of negative round cells})}$$

than calculate the concentration of peroxidase-positive white blood cells in the semen sample as follows

concentration (mill/mL) =
Proportion positive round cells x total concentration of round cells

Biochemical analysis

After completing examination, the sample then was placed in conical test tube and centrifuged at 3000 (rpm) for 10 minutes to obtain the plasma. The seminal plasma was stored in Eppendorf tubes for frozen in –20 Co for examination of antisperm antibodies (ASA), cytokine and zinc. Seminal plasma ASA and cytokine (IL-1and IL-6) had been measured by the immunological method (Enzyme-Linked-Immuno-Sorbent- Assay) employs the quantitative sandwich enzyme immunoassay technique and the zinc level in seminal plasma was measured by using a flame atomic absorption device.

Statistical analysis

Data of the current study was tabulated in Excel Microsoft Office Word software 2011, then transferred into computerized data form using two statistical software, the statistical package for social sciences (SPSS, version 23) and Medcalc Version 12.5. Variables were expressed as frequencies, percentages, or as mean and standard deviations. Statistical tests and procedures were used according to the type of the variables; chi square test used to compare categories, and independent student's t test to compare means of any two studied groups, and One Way ANOVA used when compare mean of three variables**.**

Results and Discussion

Study of semen parameters in infertile men according to type of semen viscosity

The results of current study showed a nonsignificant decrease at ($p<0.05$) in sperm concentration, progressive motility and normal sperm morphology while a nonsignificant increase at ($p<0.05$) in leucocytes in group abnormal semen viscosity as compared with group normal semen viscosity. This study agrees with Mahran and Saleh (2014) showed no statistically significant differences in sperm concentration, total sperm count and normal sperm morphology in group abnormal semen viscosity as compared with group normal semen viscosity. but showed a significant decrease of sperm motility and indicated a significant positive correlation between leukocytospermia and hyperviscosity semen

Another study showed that there was no statistically significant difference in semen volume between samples with normal viscosity compared to those with hyperviscosity, sperm concentration, progressive motility, total motility, viability, and normal morphology were significantly higher in the normal viscosity group when compared to the abnormal viscocity group (Lampiao, and Chisaka., 2020)

Table 1: Semen parameters of normal viscosity and abnormal viscosity of Infertile men

Semen parameters	Normal viscosity N=34	Abnormal viscosity N= 54	p.value
Sperm concentration (Million/ml)	30.00±29.92	26.95±18.82	0.558ns
Progressive motility (%)	33.09±24.22	26.93±21.09	0.211ns
Normal sperm morphology (%)	51.41±12.30	49.69±12.21	0.521ns
Leucocytes (Million/ml)	1.35±0.98	1.65±2.30	0.481ns

ns= Non significant differences at P≤0.05, by using Independent t-test

Study Antisperm antibody, cytokine and zinc levels in seminal plasma according to type of semen viscosity for infertile men

The tables (2) showed nonsignificant increase (P≤0.05) in antisperm antibody, interluekine 6 in abnormal semen viscosity compared to normal semen viscosity while the the study were revealed nonsignificant decrease at ($p<0.05$) in interluekine1and zinc levels in abnormal viscosity compared to normal semen viscosity. A Study by Moulik, et al (1989) showed the prevalence of anti-sperm antibodies in samples with high viscosity was significantly greater than in samples with normal viscosity. another study shown that sperm hyperviscosity correlated positively with the oxidative stress and pro-inflammatory interleukins, TNF-α or IL-6 in patients with male accessory gland infection (Castiglione, *et al.*, 2014)

Rajasekaran, *et al* (1995) was reported that an increased production of cytokines (e.g IL-6) can induce an increased reactive oxygen species (ROS) production in the

male genital tract. some studies have reported a correlation between hyper viscosity and oxidative stress (Harchegani, *et al*,2019) Mahran and Saleh (2014) reported a significant negative correlation between SHV and seminal plasma levels of fructose, ascorbic acid, zinc, and calcium. Another study that agreement with our result where showed zinc concentrations have been found to be lower in hyperviscosity samples (Andrade-Rocha,2005). another study by Mankad, *et al*, (2006) shows zinc levels were slightly lower among hyper viscosity samples compared to those with normal viscosity. these result that Agrees with our study

Seminal plasma zinc is originated primarily from the prostate gland and may reflect prostatic secretary function (Du Plessis, *et al.,*2013) Hypofunction of the prostate or seminal vesicles causes abnormal viscosity of seminal fluid (Khan, *et al.*,2011). Elzanaty, *et al* (2004) were found lower concentrations of zinc in hyper-viscous samples and this association might be due to a direct role of this metal in the process of semen viscoelasticity or rather the strong correlation to the amount of secreted Prostate-specific antigen (PSA).

Table 2: comparison of Antisperm antibody, cytokine and zinc levels between normal semen viscosity and abnormal semen viscosity for infertile men

Antisperm antibody, cytokine and zinc levels	Normal viscosity N=34	Abnormal viscosity N=54	p. value
Antisperm antibody (np / ml)	440.52±202.42	494.37±202.07	0.227ns
Interleukin 1B (IL-B) (Pg/ml)	7.21±8.54	6.32±9.15	0.649ns
Interleukin 6 (IL-B) (Pg/ml)	35.02±48.11	36.43±43.69	0.888ns
Zinc(ppm)	124.03±25.24	117.70±26.26	0.267ns

study of semen parameters, antisperm antibody, cytokine and zinc levels according to severity of semen viscosity for infertile men

The results of the study showed decline in Sperm concentration, Progressive motility and normal sperm morphology in sever semen viscosity compared to moderate semen viscosity while showed elevation in leucocytes Antisperm antibody, Interleukin 1B, Interleukin 6 and zinc levels in sever semen viscosity compared to moderate and mild semen viscosity but statistically all variation were not-significant at ($p<0.05$) The reason for the decrease in the parameters of sperm and semen and the increase in the levels of antisperm antibody and cytokines may be due to the increase in the active oxygen species and the decrease in antioxidants due to the presence of white blood cells, which is a source of free radicals.

The results of one the studies showed an increase in the concentration of white blood cells in the semen of the sever group compared to the mild and moderate group in the viscosity of semen, while reported no differences in spermatozoa motility and morphology recovery in HV group according to severe or moderate viscosity (Nosi et al, 2019)

Table 3: effect of severity semen viscosity on semen parameters for infertile men

Semen parameters	Moderate and Mild viscosity N=16	Sever viscosity N= 38	P. value
Sperm concentration (Million/ml)	31.29±19.28	24.96±18.52	0.254ns
Progressive motility (%)	33.18±21.59	24.05±20.51	0.141ns
Normal sperm Morphology (%)	51.35±12.02	48.92±12.39	0.501ns
Leucocytes (Million/ml)	1.46±1.90	1.65±1.52	0.699ns

*=significant differences at P≤0.05, ns= Non significant differences at P≤0.05, by using Independent t-test

Table 4: effect of severity semen viscosity on antisperm antibody, Interleukin 1B, Interleukin 6 and zinc levels for infertile men

Antisperm antibody, cytocine and zinc levels	Moderate and Mild viscosity N=16	Sever viscosity N= 38	P. value
Antisperm antibody (ng/ml)	476.51±210.11	533.23±183.25	0.343
Interleukin 1B (IL-B) (Pg/ml)	6.25±10.07	6.45±7.02	0.943
Interleukin 6 (IL-B) (Pg/ml)	31.73±49.31	46.65±26.32	0.245
Zinc (ppm)	119.08±29.84	114.71±16.32	0.575

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