Evaluation of Significance of Prolonged Liquefaction Time of Semen in Hypofertile Men

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ABSTRACT:

BACKGROUND:

Until now most of the references adopt a sixty-minute liquefaction time as a guideline for normality in general semen analysis. During the last twenty years of interest in these patients, we found that there is a correlation between primary hypofertility, prolonged liquefaction time and therefore delayed conception worthy for this study. This study represent a clinical biometric case series on (284) patients consulting an outpatient urology clinic with primary hypofertility during the last ten years in Mosul.

OBJECTIVE:

To signify the impact of prolonged liquefaction time of semen on fertility potential of primary hypofertile men, including its relation with the delay of conception and other semen parameters.

PATIENTS AND METHODS:

Married males who are sexually healthy supposed to have healthy partners with no pregnancy who were not using any method of contraception. Patients should give their semen for analysis in the laboratory by masturbation. All patients should have at least two separate samples to be analyzed, with not less than 7 days apart. Collected data included age in years and duration of delayed conception in months. Semen parameters collected were: volume in milliliters (ml), liquefaction time in minutes, concentration (density) in millions/milliliters, motility in percentage, and morphology in percentage.

RESULTS:

The mean age of people in our sample was 29.7 years, mean volume of semen was 2.9 ml, mean liquefaction time was 26 minutes, the mean sperm density was 32.9 millions/ml, while the mean activity percentage was 31.2%, the mean percentage of the normal sperms was 61.9% and the mean duration of delay in conception was 33.7 months. There was a very highly significant correlation of prolonged liquefaction time with impaired motility, and morphology, and a significant correlation with sperm concentration. Also there was a linear positive relationship between prolonged LT in minutes and delayed conception in months, but it was more prominent in those hypofertile men who failed to conceive for ≥ 36 months.

CONCLUSION:

The prolonged liquefaction time has a possible role as a cause of delay in conception in hypofertile men, and has a significant relationship to defects in other semen parameters.

KEY WORDS: liquefaction time, hypofertility, semen, seminal fluid analysis.

INTRODUCTION:

Infertility is defined as the inability to conceive after one year of unprotected sexual intercourse. Roughly speaking, 40% of cases involve a male contribution or factors.[1,2] The chance of a normal couple conceiving is estimated to be 20% to 25% per month, 75% by 6 months and 90% by 1 year after unprotected intercourse.[2]

Except in cases of azoospermia, the semen analysis does not allow for the definitive separation of patients into sterile and fertile groups. As semen parameters decrease in quality, the statistical chance of conception decreases but does not reach zero.[2]

Delay in conception for husbands with supposed fertile female partners, is still a big challenge in fertility centers, especially whose semen parameters are within minimal standards of adequacy which is called (primary hypo-fertility).[3,4]

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Semen analysis is the cornerstone of male infertility investigations. Eliasson[5] and Hellinga[6] pioneered the scientific basis of conventional semen analysis, and the techniques they recommended are still considered to underpin more advanced techniques.[7]

During the time of ejaculation, the spermatozoa are suspended in the secretions of prostate, seminal vesicles, bulbo-urethral glands, and other accessory glands that form a coagulum.

The specimen usually liquefies within 30 minutes. However, semen obtained from patients with congenital bilateral absence of the vas usually does not form a coagulum and is acidic. Liquefaction is aided by the proteolytic enzyme fibrinolysin, secreted by the prostate. Improper or prolonged liquefaction indicates an ejaculatory duct obstruction or poor prostatic secretion.[2]

Freshly produced semen is a coagulum that liquefies 5 to 25 minutes after ejaculation. The constituents of the semen responsible for coagulation originate in the seminal vesicles; the proteolytic enzymes that initiate liquefaction are found in the prostate. Following liquefaction, seminal fluid viscosity can be qualified. Normal viscosity is defined as occurring when the specimen can be poured drop by drop. Impaired liquefaction and increased viscosity remain equivocal causes of infertility and cannot be considered significant in the presence of a normal postcoital test.[8]

Increased viscosity has the same clinical meaning as abnormal liquefaction.[9] Some variation in macroscopic parameters (i.e., prolonged liquefaction time (LT)) is relatively common and is thought to be of little clinical significance. Semen liquefaction is thought to be due to prostatic derived proteases, including prostate-specific antigen and plasminogen activator.[1,2,9]

OBJECTIVE:

The aim of this study is to signify the presence of association between semen liquefaction time (LT), delay in conception and other semen parameters, and to identify the impact of prolonged liquefaction time on fertility potential in primary hypofertile men.

PATIENTS AND METHODS:

This is a case series study of 284 male patients presented as primary hypo-fertility during the last ten years to a private urologist clinic in the period from 1st of June 2001 to 31st of May 2011.

Semen was procured in the laboratory (one sample only) by self-induction into a clean plastic container and conventionally examined in the 4th day of abstinence.[10] All patients should have at least two separate samples to be analyzed. These samples should be not less than 7 days apart.[11,12] All samples were tested in the same laboratory. Exclusion criteria included samples brought from home, patients taking medicines especially hormones in such cases samples were repeated after 1 and 3 months from stopping the treatment. Patients with azoospermia, as well as, patients with incomplete parameters or inadequate history were also excluded.

In accordance with standardized techniques for semen analysis that have been reported, we allow the ejaculate to liquefy at 37°C, and liquefaction time is measured. Volume is measured in graduated cylinder to the nearest 0.1 ml. a small drop of semen is mounted on a microscope slide with a coverslip for evaluation of motility, forward progression, and agglutination. An aliquot of a 1:20 dilution of semen (0.95 ml distilled water and 0.05 ml of semen using a pipette) is placed on a hemocytometer for determination of sperm density and morphology.[8]

Collected data included age in years and duration of delayed conception in months. Semen parameters collected were: volume in milliliters (ml), liquefaction time in minutes, concentration (density) in millions/milliliters, motility in percentage, and morphology in percentage. Details of the activity of the sperms in the specimen includes the percentage of the motile (for those whose lab classification of motility is of two grades), the highly active motile (of three grades), but those with four grades, a straight forward progress summed with the highly active motile is taken. Repeated visits of the same patient were considered as one case.

Simple percentages, standard deviation (SD) were used, Pearson correlation was calculated to find the presence of correlation between duration of delayed conception and values of semen analysis parameters. Un-paired t- test was used to ascertain the presence of significant differences of parameters between patients with prolonged liquefaction time (>30 minutes) and those with ≤20 minutes. P-value ≤0.05 was considered significant. Oral consents were taken from patients to be involved in this study and ethical agreements were registered in the Directorate of Health in Ninawa.

RESULTS:

Table 1 describes semen analysis parameters among study cases. The mean age was 29.7 years, mean volume of semen was 2.9 ml, mean liquefaction time was 27.1 minutes, and the

concentration was 33.5 million/ml; while the mean motility percentage was 32.3%. Two thirds of sperms (62.2%) were morphologically normal and the mean duration of delayed conception was 33.7 months.

Table 1: Semen analysis parameters among study cases (n=284).

Parameter (n=284)	Liquefaction time (minutes)	Concentration (million/ml)	Motility %	Morphology %	Delay in conception (Months)
Minimum	3	0.3	0.8	5.0	1.0
Maximum	120.0	130.0	85.0	95.0	288.0
Arithmetic Mean	27.1	33.5	32.3	62.2	33.7
Standard Deviation	13.4	23.7	18.8	16.7	43.9

Table 2 presents semen analysis parameters in two groups of patients: the 1st group are those with liquefaction time more than half an hour (sixty-five patients), the 2nd group includes patients whose liquefaction time is ≤20 minutes (one hundred nineteen patients). The mean liquefaction time of the 1st group was significantly higher than that of the 2nd group (45.86±17.48 minutes versus 17.61±3.07minutes; P=0.000). Considering motility and morphology percentages, similar findings were reported (P=0.000) each. Unfortunately, the mean duration of delay in conception showed no significant difference among the two groups.

Table 2: Semen analysis parameters among patients with delayed and normal liquefaction.

Parameters	Liquefaction time >30min (n=65) Mean ±SD (1st group)	Liquefaction time ≤20min (n=119)Mean ±SD (2nd group)	P-value
Age (years)	29.92±7.13	28.71±5.6	0.203
Liquefaction time (minutes)	45.86±17.48	17.61±3.07	0.000
Sperm concentration (millions/ml)	30.84±21.83	39.93±26.23	0.018
Motility percentage (%)	24.61±15.89	37.64±20.18	0.000
Morphology Percentage (%)	56.37±16.83	66.82±14.18	0.000
Delay in conception (months)	29.21±34.44	27.19±31.46	0.695

Table 3 presents the degree of correlation between semen analysis parameters and duration of delay in conception in months. Morphology in percent is the unique parameter which shows a significant positive correlation with duration in conception (R=0.116, P=0.045).

Table 3: Correlation between semen analysis parameters and duration of delay in conception (months).

Parameters	R	P –value
Liquefaction time (n=284)	0.019	0.747
Liquefaction time in those with 36months delay in conception (n=91)	0.045	0.668
Motility (%)	-0.096	0.099
Morphology (%)	0.116	0.045

Figure 1 and 2 show that there is a linear positive relationship between prolonged LT in minutes and delayed conception in months, but it was more prominent in those hypofertile men who failed to conceive for ≥ 36 months (figure 2).

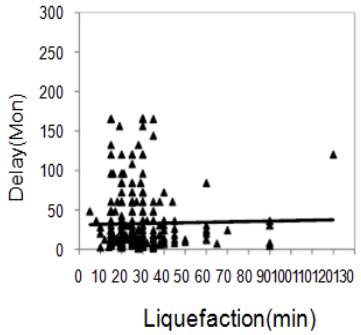

Figure 1: Correlation between the liquefaction time in minutes and delay of conception in months.

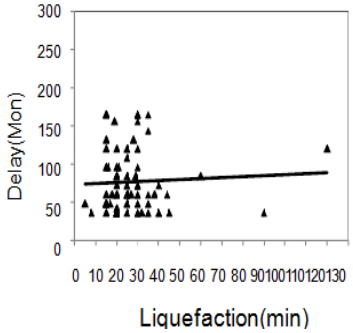

Figure 2: Correlation between the delay in conception for more than 36 months with their liquefaction time.

DISCUSSION:

Several studies showed the minimal standards of adequacy of semen analysis which include [volume 1.5mL, concentration 15 million per ml, total motility 40%, progressive motility 32%, morphology > 30% WHO normal forms, no agglutination or increased viscosity].[1,2,9,13,14,15,16]

There is discrepancy between the expected chance of normal couple conceiving and the above mentioned parameters, this space which is called (primary hypo-fertility), may not be due to one of them.

Until now there is no unified opinion about the normal time of liquefaction as a parameter in general semen analysis, and there was no definite opinion that liquefaction time is an effective factor in determining fertility in males.[1,2,9,16]

Some consider the normal liquefaction time as being five minutes to one hour, others consider it to be even more than one hour; especially if the post coital test(P.C.T.) revealed the presence of adequate number of motile sperms in the cervical mucus after ejaculation, they also consider it as a normal finding in general semen analysis.[1,2,9,16] It is not even mentioned in the lower reference limits for semen characteristics.[16]

The statistical analysis showed that there is an increase of mean liquefaction time in patients who have the delay in conception of more than 36 months. In Our study Statistical analysis showed that there is a definite effect of liquefaction time on the delay of pregnancy, and there was directly proportional linear relationship between prolonged LT and delayed conception especially for those of more than 36 months delay in pregnancy, although it was not statistically significant.

But when two groups of patients seminal parameters were correlated according to their liquefaction time, the 1st group of ≤ 20 minutes and others of more than 30 minutes effect. There was a very highly significant correlation of prolonged liquefaction time with impaired motility, and morphology, and a significant correlation with sperm concentration (table 2). There is also significant negative correlation between percentage of normal sperm morphology and delay of conception. So we may guess an indirect effect of prolonged liquefaction on delay in conception through abnormal morphology, deranging the motility and hence fertilization.

Mandal A and Bhattacharyya AK (1987) supposed that during comparison of presumptively fertile and infertile ejaculates showed significant variations in their amount liquefaction time. The study suggested a possible relationship between the coagulation-liquefaction property of human ejaculates and their semen quality including sperm count, motility and semen volume.[17]

At 1985, they also investigated the relationship between spontaneous liquefaction and the characteristics of ejaculated human semen. Human ejaculates were classified into 3 distinctgroups depending on their liquefaction time, and the groups were characterized physico-chemically. The liquefaction time also revealed a low but significant negative correlation with semen volume. It is concluded that an individual's ejaculatory characteristics can be evaluated simply by determining its liquefaction time.[18]

Mandal A and Bhattacharyya AK (1988) concluded that relative decrease in the prostatic activity with respect to that of the seminal vesicles appears to be the cause of slow-liquefaction.[19] At 1987, they also concluded that the material

characteristics of human ejaculate can be approximated simply by determining its degree of coagulation or liquefaction time.[20]

Xang XF etal (2012)[21], Sun XZ et al (2011)[22], Zhang XD et al (2009)[23], and Xiong GB et al (2009)[24] proved a statistically significant evidence that treatment of prolonged LT with medications that shorten LT showed obvious improvement in sperm motility and concentration (sperm density).

All these results supported our conclusion that there is a significant correlation between prolonged time of liquefaction and defects in other parameters of semen analysis that measured by the conventional methods; which could be the cause behind the significant delay in conception in those hypofertile men with long infertility duration.

CONCLUSION:

The study suggested a possible role of prolonged LT as a cause of infertility and delay in conception in hypofertile men with long time of failure to conceive, and documented its relationship with other defects in semen parameters, where it has highly significant correlation with low motility and abnormal morphology, and significant relationship with low sperm density, which could contribute to indirect effect of prolonged LT on fertility potential of those hypofertile men.

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