

Standardization and Quality Control for Determination of Fructose in Seminal Plasma

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In this age of evidence-based medicine, there is nothing more important than the quality of lab tests (Westgard and Darcy, 2004). However, reliability of semen analysis has been suspected for lack of standardization, and significant variation exists within the same laboratory and among different laboratories (Chong et al, 1983; Keel et al, 2000; Keel et al, 2002b; Brazil et al, 2004; Keel, 2004). In recent years, andrologists have placed greater emphasis on standardization of semen analysis (Keel, 2002a; Huang and Li, 2005). At present, quality controls for determinations of sperm concentration, motility, morphology, and antisperm antibodies have been emphasized widely (Cooper et al, 2002; Toft et al, 2005). However, few reports exist on standardization and quality control for biochemical analysis of seminal plasma (Lu et al, 2007). Therefore, we attempted to establish standardization and quality control for the determination of fructose concentration in seminal plasma.

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Andrology Lab Corner*

Determination of seminal fructose concentration has been used in examination of obstructive azoospermia and inflammation of male accessory glands (Carpino et al, 1997; Manivannan et al, 2005). Inflammation may lead to atrophy of the seminal vesicle and low seminal fructose concentration. When ejaculatory ducts are blocked, fructose concentration in seminal plasma usually decreases, and may become undetectable (Coppens, 1997). Additionally, determination of fructose concentration in seminal plasma is useful to auxiliary diagnosis of obstructive and nonobstructive azoospermia. Seminal fructose concentration in nonobstructive azoospermia is usually higher than or equal to that in males of normal fertility (Buckett and Lewis-Jones, 2002). However, fructose concentration in seminal plasma of patients with obstructive azoospermia is usually absent or significantly lower than that in men of normal fertility (Manivannan et al, 2005). Absence of seminal fructose is also found in patients with congenital vas deferens-seminal vesicle developmental defect (Kise et al, 2000; Kumar et al, 2005). Determination of fructose concentration has also been used for auxiliary diagnosis of retrograde ejaculation, where fructose concentration in urine from the bladder after ejaculation was detected.

Thus, the accuracy of fructose concentration determination in seminal plasma is very important. To guarantee accuracy of fructose determination, assays of fructose in seminal plasma must be standardized, and quality control should be involved. Here, we report effects of standard fructose solution, centrifugation velocity, standing time of semen and seminal plasma, freezing-thawing, and chymotrypsin exposure on detection of fructose concentrations in seminal plasma. Lastly, we compare fructose concentrations determined by 2 technicians.

Materials and Methods

Reagents and Instruments—Semen analysis was performed with a computer-assisted sperm analysis (CASA) system (WLJY-9000; Beijing Weili New Century Science & Tech Dev Co, Ltd, Beijing, China). Spectrophotometer (721 type) was provided by Shanghai Analysis Instrument Factory (Shanghai, China). KDC-1044 Low Speed Tabletop Centrifuge was provided by USTC Chuangxin Corporation Limited ZONKIA Branch

(Hefei, China). Both low ($[18 \pm 2.5] \times 10^6/\text{mL}$) and high ($[35 \pm 5] \times 10^6/\text{mL}$) precalibrated standard latex bead solutions (Hamilton Thorne Biosciences, Beverly, Mass) were used as quality control solutions for sperm concentration. Chymotrypsin for injection (5 mg/4000 U, Lot 0506042), dissolved with 0.5 mL of normal saline to be 8000 U/mL of stock solution, was provided by the Department of Pharmacy, Nanjing Jinling Hospital. D-fructose (analytical reagent), prepared to be 2.78 mmol/L of stock solution with distilled water, was the product of the Chemical Reagent Limited Company, Shanghai Guoyao Bloc (Shanghai, China).

Sample Resource—Semen samples, collected by masturbation, were from outpatients of the Department of Andrology, Nanjing Jinling Hospital. All subjects signed informed consents to participate in this study, which was approved by the Ethics Committee of Nanjing Jinling Hospital. After liquefaction, routine analysis of semen samples was performed, including assessment of semen volume, pH, sperm concentration, motility, and grade a and b motility, and the remaining samples were applied to our investigation.

Monitoring Stability of Standard Fructose Solution—Standard fructose solution (0.28 mmol/L) was prepared with stock solution by adding distilled water and stored at 4°C, then stability was continuously monitored for 35 days by the resorcinol method. One mL of standard fructose solution, 1 mL of resorcinol solution (8.47 mmol/L), and 3 mL of HCl (10 mol/L) were mixed and maintained at 90°C for 10 minutes. Standard fructose solution was replaced with distilled water as blanks. Lastly, the optical density (OD) values were read at 490 nm against blanks.

Determination of Fructose Concentration in Seminal Plasma with Different Standing Times—Twenty samples of fresh liquefied semen were randomly chosen and centrifuged at $3000 \times g$ for 15 minutes. Then fructose concentrations in seminal plasma, after standing at room temperature (20–25°C) for 0, 2, 4, or 6 hours, were detected as previously described (Huang and Xu, 1999). Fructose in seminal plasma reacts with resorcinol in concentrated HCl solution to form a red compound under heating. In detail, 0.1 mL of fresh seminal plasma sample was mixed with 2.9 mL of distilled water. Then 0.5 mL of $\text{Ba}(\text{OH})_2$ solution (0.15 mol/L) and 0.5 mL of ZnSO_4 solution (0.175 mol/L) were added, mixed, and allowed to stand for 5 minutes to remove seminal proteins. After centrifugation at $3000 \times g$ for 15 minutes, 1 mL of the supernatant was collected for determination of fructose level. The supernatant was replaced with standard fructose solution (0.28 mmol/L) to serve as standard and replaced with distilled water as blanks. Subsequently, 1 mL of resorcinol solution (8.47 mmol/L) and 3 mL of HCl (10 mol/L) were added

into tubes and maintained at 90°C for 10 minutes. Lastly, absorbance values were read at 490 nm against blanks. Fructose concentration in seminal plasma was expressed as mmol/L: absorbance value of test tube/absorbance value of standard tube $\times 11.12$.

Determination of Fructose Concentration in Seminal Plasma Obtained From Centrifuged Semen After Different Standing Times—Forty-eight samples of fresh liquefied semen were randomly chosen and divided into triplicate. One was centrifuged immediately (0 hours) at $3000 \times g$ for 15 minutes, and the other 2 were centrifuged in a similar manner after standing at room temperature (20–25°C) for 2 hours or 4 hours. Fructose concentration in seminal plasma was detected by the described method.

Determination of Fructose Concentration in Sperm Suspension—Ten fresh samples of semen were randomly chosen and washed twice with normal saline. Sperm suspensions of various concentrations were prepared for detection of fructose concentration.

Determination of Fructose and Sperm Concentrations in Seminal Plasma Obtained by Centrifugation at Different Velocities—Each of 68 samples of fresh semen was divided into 2 aliquots, one centrifuged at $1000 \times g$ for 10 minutes and the other at $3000 \times g$ for 15 minutes. Fructose and sperm concentrations in seminal plasma were detected by the resorcinol method and CASA system with Makler chamber, respectively. Precalibrated standard latex bead solutions were used as quality control solutions to provide accuracy of sperm count. In detail, a drop of 5- μL standard bead solution or seminal plasma obtained at $1000 \times g$ for 10 minutes and $3000 \times g$ for 15 minutes was put on chambers. To ensure the accuracy of bead concentration or sperm counting, more than 10 fields or a minimum of 200 beads/sperm were counted for each chamber.

Effect of Chymotrypsin on Detection of Fructose Concentration in Seminal Plasma—Each of 72 samples of fresh liquefied semen was divided into 2 aliquots, one without chymotrypsin and the other with 1% (v/v) of chymotrypsin (8000 U/mL). Samples were incubated at 37°C for 30 minutes, centrifuged at $3000 \times g$ for 15 minutes, and analyzed for seminal plasma fructose concentrations.

Detection of Fructose Concentration in Nonliquefied Semen Samples—Thirty-nine samples of fresh nonliquefied semen, obtained 1 hour after collection, were incubated with 1% (v/v) of chymotrypsin (8000 U/mL) at 37°C for 30 minutes and centrifuged at $3000 \times g$ for 15 minutes. Fructose concentration in seminal plasma was detected.

Detection of Fructose Concentration in Seminal Plasma after Freezing-Thawing—An additional 2 samples of seminal plasma, obtained by centrifugation at

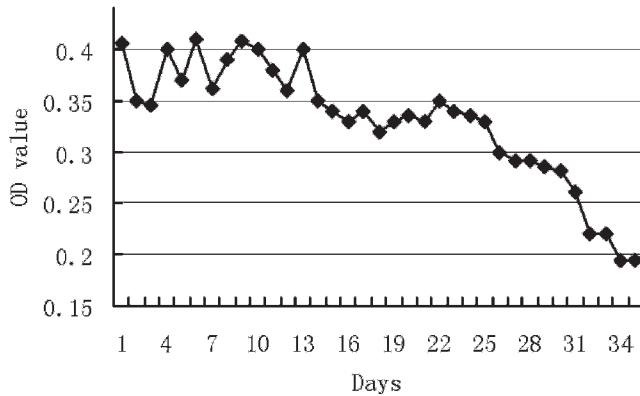


Figure 1. OD value change of standard fructose solution with time.

3000 × g for 15 minutes without sperm residue, as confirmed by CASA, were frozen. Every other day, both samples were thawed to determine the fructose concentration. The remains were then refrozen for subsequent tests. The process of thawing and refreezing was repeated 10 times within 20 days.

Determination of Fructose Concentration in Seminal Plasma by 2 Technicians—Ten samples of fresh semen were centrifuged at 3000 × g for 15 minutes, and resultant seminal plasma samples were divided into 2 aliquots to be blindly determined by 2 technicians.

Statistical Analysis—Data were expressed as mean ± SD and analyzed with SPSS 11.0 software (SPSS Inc, Chicago, Ill). Comparisons of fructose concentrations in seminal plasma with different standing durations, obtained from centrifuged semen, with or without chymotrypsin were analyzed by paired *t* test. Comparisons between fructose concentrations and between sperm concentrations in seminal plasma obtained by centrifugation at different velocities were also analyzed by paired *t* test. Comparison of fructose concentrations detected by 2 technicians was shown with Bland and Altman plot. Correlation between decrease of fructose concentration in seminal plasma obtained from centrifuged semen after different standing times and motile sperm concentration was analyzed by Pearson's correlation. Comparison of fructose concentration between liquefied and nonliquefied semen samples was analyzed by independent-sample *t* test. Level of significance was set at *P* less than .05.

Results

Monitoring Stability of Standard Fructose Solution—The stability of standard fructose solution was monitored for 35 days successively, as indicated in Figure 1. The variation of optical density (OD) was wide within the first 2 weeks after preparation of standard fructose solution. The OD value decreased slightly and remained

Table 1. Comparison of fructose concentrations (Mean ± SD) in seminal plasma standing for 0, 2, 4, or 6 hours after centrifugation of semen

Group	n	Fructose (mmol/L)
0 h	20	16.01 ± 5.84
2 h	20	15.96 ± 5.56
4 h	20	15.79 ± 5.50
6 h	20	16.23 ± 5.39

stable during the following 2 weeks. About 1 month later, the OD value decreased quickly.

Effects of Seminal Plasma with Different Standing Times on Fructose Concentration—Fructose concentrations in seminal plasma standing for 0, 2, 4, or 6 hours after centrifugation of semen samples are shown in Table 1, indicating that there was no significant difference of fructose concentration in seminal plasma with different standing times (*P* > .05).

Effects of Semen with Different Standing Times on Fructose Concentration—Fructose concentrations in seminal plasma obtained from centrifuged semen after standing for 0, 2, or 4 hours are shown in Table 2. Fructose concentration decreased with length of standing time. Fructose concentration after standing for 2 hours was significantly lower than that after 0 hours, and much more significantly lower after 4 hours than after 0 or 2 hours.

It was also found that the decrease of fructose level after standing semen was correlated with sperm concentration and motility. Thus, the slope rate (K) was calculated, representing the decrease of each seminal fructose using Excel to plot the value of fructose level at 0, 2, and 4 hours. Meanwhile, each of the motile sperm concentrations was calculated by motility × sperm concentration. Subsequently, Pearson's correlation analysis was performed between K and motile sperm concentration, showing that the decrease of seminal fructose concentration had significantly positive correlation with motile sperm concentration (*r* = .374, *P* = .009; Figure 2).

Fructose Concentration in Sperm Suspension—Fructose concentrations were all 0 for 10 samples of sperm

Table 2. Comparison of fructose concentrations (Mean ± SD) in seminal plasma obtained from centrifuged semen after standing for 0, 2, or 4 hours

Group	n	Fructose (mmol/L)*
0 h	48	15.73 ± 6.56
2 h	48	15.07 ± 6.56 ^a
4 h	48	14.73 ± 6.51 ^{b,c}

*Compared with 0 hours group, ^a*P* = .004, ^b*P* = .000; compared with 2 hours group, ^c*P* = .006.

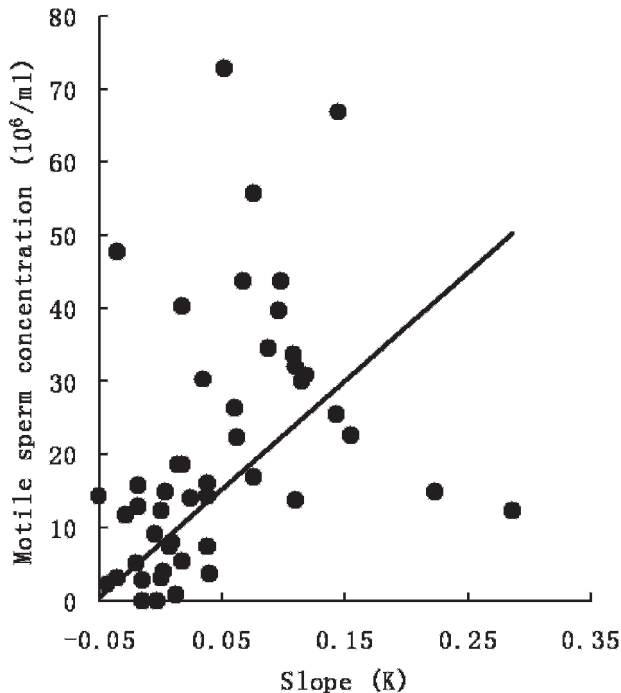


Figure 2. Correlation between the decrease of fructose concentrations in semen (K) and motile sperm concentration ($r = .374$).

suspension, indicating that fructose in sperm suspension was undetectable.

Comparison of Fructose and Sperm Concentrations in Seminal Plasma Obtained by Centrifugation at Different Velocities and Times—Fructose concentrations in

seminal plasma obtained by centrifugation at $1000 \times g$ for 10 minutes or $3000 \times g$ for 15 minutes were (15.96 ± 7.62) mmol/L and (16.34 ± 8.06) mmol/L, respectively, and not significantly different ($t = -1.369$, $P = .176$). After centrifugation at $1000 \times g$ for 10 minutes, there were 92.64% (63/68) of seminal plasma containing sperm with an average concentration of $2.81 \times 10^6/\text{mL}$ (range from 0 to $19.40 \times 10^6/\text{mL}$). Conversely, centrifugation at $3000 \times g$ for 15 minutes resulted in 44.12% (30/68) of seminal plasma containing sperm with average concentration of $0.41 \times 10^6/\text{mL}$ (range from 0 to $5.67 \times 10^6/\text{mL}$), which was a significant difference compared to centrifugation at $1000 \times g$ for 10 minutes ($t = 7.546$, $P < .001$; Figure 3).

Effect of Chymotrypsin on Seminal Fructose Determination—Fructose concentrations in seminal plasma with or without chymotrypsin are shown in Table 3, which indicates that chymotrypsin had no apparent effect on seminal fructose determination.

Seminal Fructose Concentrations in Nonliquefied Semen Samples—After 1% (v/v) of chymotrypsin (8000 U/mL) was added to 39 samples of semen, liquefaction was improved; seminal fructose concentration is shown in Table 4. While chymotrypsin may improve liquefaction of nonliquefied semen, there was no significant difference in fructose concentration between liquefied and nonliquefied samples.

Effect of Freezing-Thawing on Seminal Fructose Concentration—Two samples of semen were stored at

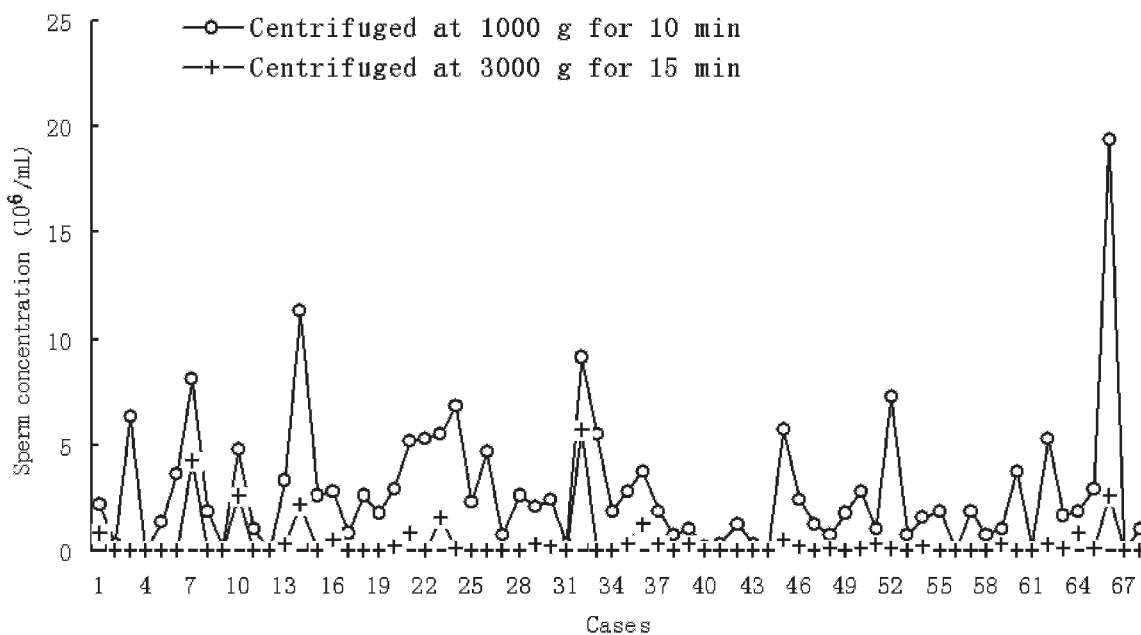


Figure 3. Comparison of sperm concentrations in seminal plasma obtained by centrifugation at $1000 \times g$ for 10 minutes or $3000 \times g$ for 15 minutes.

Table 3. Comparison of fructose concentrations (Mean ± SD) in seminal plasma samples with or without chymotrypsin

Group	n	Fructose (mmol/L)	t	P
With chymotrypsin	72	17.29 ± 8.23		
Without chymotrypsin	72	17.07 ± 7.95	0.867	.389

−20°C (not frost-free) for 20 days, and the seminal fructose concentration was detected every other day. Results are shown in Figure 4. Fructose concentration in sample 1 was (25.96 ± 1.33) mmol/L with 5.05% coefficient of variation (CV), and (15.68 ± 1.06) mmol/L in sample 2 with 6.61% CV, suggesting that freezing-thawing had no apparent effect on seminal fructose concentration.

Comparison of Seminal Fructose Concentrations Determined by 2 Technicians—Ten samples of seminal fructose concentrations detected by 2 technicians are shown in Figure 5. Seminal fructose concentrations detected by 2 technicians were (16.12 ± 5.78) mmol/L and (16.01 ± 5.78) mmol/L, respectively, and there was no significant difference between their results (P = .643).

Discussion

The normal function of seminal vesicle is essential for sustaining fertility. When the function of seminal vesicle decreases, semen coagulation, sperm motility, stability of sperm chromatin, and semen immunoprotection are affected (Montagnon et al, 1990). One of the most important markers for the seminal vesicular function is the concentration of fructose in seminal plasma.

Fructose is a main carbohydrate source in seminal plasma and necessary for sperm motion (Buckett and Lewis-Jones, 2002; Santiani et al, 2005). The measurement of seminal fructose has been used in most laboratories. Therefore, the World Health Organization manual recommends measurement of seminal fructose as a marker of seminal vesicular function (World Health Organization, 1999). Methods for determination of seminal fructose mainly include gas chromatography, indole coloration, and resorcinol coloration. In particular, the resorcinol method has been used widely in clinical andrology laboratories for its simplicity of operation, high specificity, and no need for special instrument. In the present study, 2 technicians were responsible for the determination of 10 samples of

Table 4. Comparison of fructose concentrations (Mean ± SD) between liquefied and nonliquefied semen samples

Group	n	Fructose (mmol/L)	t	P
Liquefied	86	16.96 ± 8.06		
Nonliquefied	39	15.35 ± 8.56	1.032	.304

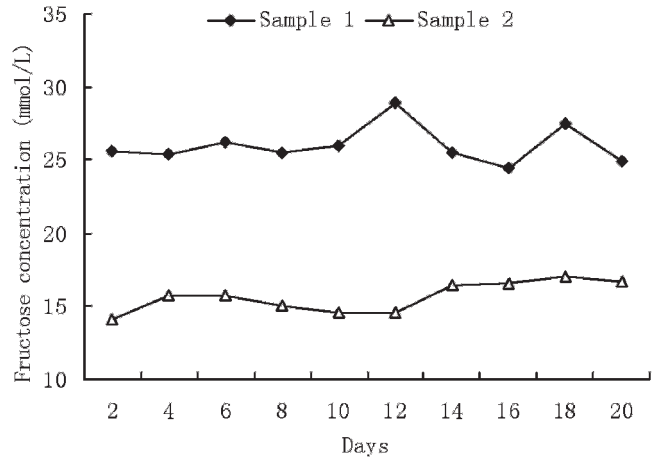


Figure 4. Fructose concentrations of samples 1 and 2 during 20 days.

seminal fructose, showing no significant difference between their results (P = .643). This supports the notion that the resorcinol method used in determination of seminal fructose is now feasible for general technicians working in andrology laboratories.

In routine assays of semen, we found that standard fructose solution tended to be stable within 15 days after preparation. Thus, we monitored the OD value change of standard fructose solution. Results showed that there was a large variance of OD values within the first 2 weeks and that OD values remained relatively stable within the subsequent 2 weeks, falling quickly from 0.30 to 0.19 within the last 10 days. Fructose, a polyhydroxy ketose, has dissymmetry carbon atoms with optical activity, which is mutarotation in water (Tan et al, 2005). About 2 weeks later, 5 possible isomers, including types α (levo- and dextro-rotation), β (levo- and dextro-rotation), and straight chain, achieved a balance, in

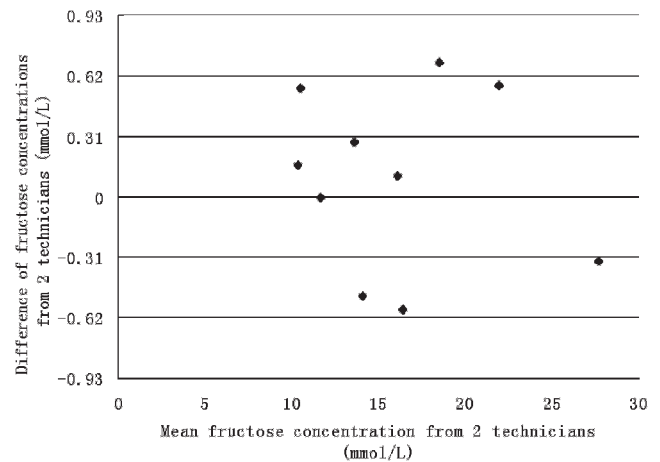


Figure 5. Bland and Altman plot of seminal fructose concentrations determined by 2 technicians. SD = 0.31. The range of 95% CI is from −0.62 to 0.62.

accordance with specific rotation achieving a balance with relative stability. Therefore, OD values of standard fructose solution were relatively stable, with a slight variance 2 weeks after preparation. About 4 weeks later, with the weak nucleophilic reagent of water, ketose was transformed into aldose and also achieved a balance within a period of time. As resorcinol and concentrated hydrochloric acid react with ketose to form more red compound than with aldose, OD value would decrease rapidly within the last 10 days. For these reasons, in order to gain accurate and reliable seminal fructose concentration, standard fructose solution should be stored for 2 weeks before use and discarded when the decrease of OD value appears.

Our data showed that there was no obvious difference of fructose concentrations in seminal plasma standing for 0, 2, 4, or 6 hours. Next the change of seminal fructose within 4 hours of standing of semen was observed, showing that seminal fructose concentration decreased with length of standing duration. The concentration of fructose at 2 hours was significantly lower than that measured immediately, and at 4 hours significantly lower than at 2 hours. Furthermore, the decrease of fructose concentration was significantly positive correlated with motile sperm concentration ($r = .374$, $P = .009$), verifying the demonstrations that motile sperm in vitro can unceasingly consume fructose, and evaluation of seminal vesicular function should be performed within 3 hours after ejaculation (Rui et al, 1986). We suggest that standing time of semen should be standardized and that semen should be centrifuged immediately after liquefaction and separated into seminal plasma and sperm, to ensure accuracy of seminal fructose determination.

Fructose concentrations in seminal plasma obtained by centrifugation at $1000 \times g$ for 10 minutes or $3000 \times g$ for 15 minutes were also observed, showing that there was no significant difference of fructose concentrations ($t = -1.369$, $P = .176$) but there was significant difference of sperm concentrations ($t = 7.546$, $P = .000$) in seminal plasma obtained by centrifugation at different velocity. When centrifugation velocity increased, seminal fructose concentration tended to increase, possibly due to apparently decreased sperm concentration in seminal plasma. Our investigation of sperm suspension showed that sperm did not contain fructose. So rudimentary sperm in seminal plasma obtained at low-velocity centrifugation would occupy some volume, leading to the decrease of actual volume of seminal plasma and then a decrease of fructose concentration. This indicates that though centrifugation velocity had no statistically significant effect on the determination of seminal fructose concentration, centrifugation velocity should not be below $3000 \times g$ for

15 minutes to gain "pure" seminal plasma and comparable results of seminal fructose detection among different laboratories.

Nonliquefied semen samples are often found in andrology laboratories. To improve the liquefaction of semen samples, chymotrypsin, extranase, or trypase are often added to semen samples (World Health Organization, 1999). Among them, chymotrypsin is inexpensive and used most widely. Therefore, we added chymotrypsin to liquefied semen samples and compared fructose concentrations in seminal plasma with or without chymotrypsin, showing that chymotrypsin had no significant influence on the determination of seminal fructose ($P = .389$). In addition, liquefaction of 39 nonliquefied semen samples was improved apparently after adding chymotrypsin, without any viscin thread when sampling. Meanwhile, there was no significant difference of seminal fructose concentration between liquefied and nonliquefied semen samples ($P = .304$). Findings indicated that nonliquefied semen samples with addition of 1% (v/v) chymotrypsin (8000 U/mL) in advance and incubated at 37°C for 30 minutes could be involved in detection of fructose concentration.

Refrigeration of seminal plasma is necessary for establishing quality control of seminal fructose determination. Therefore, we investigated fructose concentrations in seminal plasma stored at -20°C for 20 days successively, with 5.05% and 6.61% of CVs for 2 samples of seminal plasma respectively, indicating that freezing-thawing had no influence on seminal fructose concentration, and that freezing-thawing seminal plasma could serve as a quality control product for determination of seminal fructose concentrations.

Internal quality control (IQC) and external quality control (EQC) has been routine in laboratories performing clinical chemistry or endocrine analysis (Neuwinger et al, 1990). In recent years, many andrologists have been trying to introduce EQC into andrology laboratories (Auger et al, 2000). In order to establish IQC and EQC programs successfully in andrology laboratories, the prerequisite is standardization of technical procedures, which requires objective methods of semen parameters determination. Now such methods are being established and evaluated. With the help of strict IQC and EQC programs, results from different laboratories will be more comparable in the future.

In conclusion, the resorcinol method to determine seminal fructose concentration can now be accepted, and determination of seminal fructose concentration should be standardized. First, standard fructose solution should be kept for 2 weeks at 4°C before use, and the valid period for use is about 2 weeks. Next, semen samples should be centrifuged and separated into sperm and seminal plasma immediately after liquefaction; otherwise,

motile sperm will consume fructose. Thus, the standing time of semen should also be standardized. For non-liquefied semen samples, 1% (v/v) of chymotrypsin (8000 U/mL) can be added in advance and incubated at 37°C for 30 minutes to aid in liquefaction, and then for the detection of fructose concentration. Although centrifugation velocity had little influence on the determination of seminal fructose concentration, remnant sperm or other noncell components in seminal plasma may mildly affect the fructose level. Lastly, freezing-thawing had no apparent effect on seminal fructose concentration, indicating that frozen seminal plasma could serve as a quality control product for the determination of seminal fructose concentration among different laboratories.

References

- Auger J, Eustache F, Ducot B, Blandin T, Daudin M, Diaz I, El Matribi S, Gony B, Keskes L, Kolbezen M, Lamarte A, Lornage J, Nomal N, Pitaval G, Simon O, Virant-Klun I, Spira A, Jouannet P. Intra- and inter-individual variability in human sperm concentration, motility and vitality assessment during a workshop involving ten laboratories. *Hum Reprod.* 2000;15:2360–2368.
- Brazil C, Swan SH, Drobnis EZ, Liu F, Wang C, Redmon JB, Overstreet JW. Standardized methods for semen evaluation in a multicenter research study. *J Androl.* 2004;25:635–644.
- Buckett WM, Lewis-Jones DI. Fructose concentrations in seminal plasma from men with nonobstructive azoospermia. *Arch Androl.* 2002;48:23–27.
- Carpino A, De Sanctis V, Siciliano L, Maggiolini M, Vivacqua A, Pinamonti A, Sisci D, Ando S. Epididymal and sex accessory gland secretions in transfusion-dependent beta-thalassemic patients: evidence of an impaired prostatic function. *Exp Clin Endocrinol Diabetes.* 1997;105:169–174.
- Chong AP, Walters CA, Weinrieb SA. The neglected laboratory test. The semen analysis. *J Androl.* 1983;4:280–282.
- Cooper TG, Bjorndahl L, Vreeburg J, Nieschlag E. Semen analysis and external quality control schemes for semen analysis need global standardization. *Int J Androl.* 2002;25:306–311.
- Coppens L. Diagnosis and treatment of obstructive seminal vesicle pathology [in French]. *Acta Urol Belg.* 1997;65:11–19.
- Huang YF, Li PS. Urgent need for the standardization of semen analysis among clinical andrology laboratories [in Chinese]. *Zhonghua Nan Ke Xue.* 2005;11:83–84.
- Huang YF, Xu RJ, eds. *Nan Ke Zhen Duan Xue* [in Chinese]. Shanghai, China: Shanghai 2nd Military Medical University Press. 1999;219–221.
- Keel BA. How reliable are results from the semen analysis? *Fertil Steril.* 2004;82:41–44.
- Keel BA. Quality control, quality assurance, and proficiency testing in the andrology laboratory. *Arch Androl.* 2002a;48:417–431.
- Keel BA, Quinn P, Schmidt CF Jr, Serafy NT Sr, Serafy NT Sr, Schalue TK. Results of the American association of bioanalysts national proficiency testing programme in andrology. *Hum Reprod.* 2000;15:680–686.
- Keel BA, Stembridge T, Pineda G, Serafy NT Sr. Lack of standardization in performance of the semen analysis among laboratories in the United States. *Fertil Steril.* 2002b;78:603–608.
- Kise H, Nishioka J, Satoh K, Okuno T, Kawamura J, Suzuki K. Measurement of protein C inhibitor in seminal plasma is useful for detecting agenesis of seminal vesicles or the vas deferens. *J Androl.* 2000;21:207–212.
- Kumar R, Thulkar S, Kumar V, Jagannathan NR, Gupta NP. Contribution of investigations to the diagnosis of bilateral vas aplasia. *ANZ J Surg.* 2005;75:807–809.
- Lu JC, Chen F, Xu HR, Huang YF, Lu NQ. Preliminary investigations on the standardization and quality control for the determination of acid phosphatase activity in seminal plasma. *Clin Chim Acta.* 2007;375:76–81.
- Manivannan B, Bhande SS, Panneerdoss S, Sriram S, Lohiya NK. Safety evaluation of long-term vas occlusion with styrene maleic anhydride and its non-invasive reversal on accessory reproductive organs in langurs. *Asian J Androl.* 2005;7:195–204.
- Montagnon D, Valtat B, Vignon F, Koll-Back MH. Secretory proteins of human seminal vesicles and their relationship to lipids and sugars. *Andrologia.* 1990;22:193–205.
- Neuwinger J, Behre HM, Nieschlag E. External quality control in the andrology laboratory: an experimental multicenter trial. *Fertil Steril.* 1990;54:308–314.
- Roy S, Banerjee A, Pandey HC, Singh G, Kumari GL. Application of seminal germ cell morphology and semen biochemistry in the diagnosis and management of azoospermic subjects. *Asian J Androl.* 2001;3:55–62.
- Rui H, Morkas L, Purvis K. Time- and temperature-related alterations in seminal plasma constituents after ejaculation. *Int J Androl.* 1986;9:195–200.
- Santiani A, Huanca W, Sapana R, Huanca T, Sepulveda N, Sanchez R. Effects on the quality of frozen-thawed alpaca (*Lama pacos*) semen using two different cryoprotectants and extenders. *Asian J Androl.* 2005;7:303–309.
- Tan ZH, Jin SH, & Li N, eds. *Basic Organic Chemistry* [in Chinese]. Beijing: Science and Technology Literature Press; 2005;538.
- Toft G, Rignell-Hydbom A, Tyrkiel E, Shvets M, Giwercman A. Quality control workshop in standardization of sperm concentration and motility assessment in multicentre studies. *Int J Androl.* 2005;28:144–149.
- Westgard JO, Darcy T. The truth about quality: medical usefulness and analytical reliability of laboratory tests. *Clin Chim Acta.* 2004;346:3–11.
- World Health Organization. *Laboratory Manual for the Examination of Human Semen and Sperm-Cervical Mucus Interaction.* 4th ed. Cambridge, United Kingdom: Cambridge University Press; 1999;1–10.