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A new method for human semen glucose concentration evaluation

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Abstract

A UV-VIS spectrophotometric method was developed for fast human semen glucose concentration evaluation. Thirty human sperm samples, known to be with normozoospermia, were considered for measurements, and a simple method to analyze the concentration of glucose in human sperm was developed. Our results show that human semen glucose concentration measurements are feasible using a UV-VIS spectrophotometer. We determined the average glucose concentration in normozoospermic human semen as 47.17±4.13 mg/100mL. Though the sensitivity of the assay is similar to other chemical reagents techniques, such as glucose oxidase (GO), this assay allows an added flexibility in working range and instrumentation

Keywords: spectrophotometric, semen, normozoospermic, UV-VIS

Introduction

A number of technologies such as the assessment of glucose (Glu), lactate, pyruvate, and amino acid metabolism, proteomic profiling, evaluation of oxygen consumption, and most recently, examination of the metabolome are under investigation (J. G. BROMER & al. [1]).
Measurements of Glu, Urea, Triglyceride, Total Protein, Albumin, Hemoglobin and Hematocrit in whole blood can be done using noninvasive spectroscopic methods (M. K. A. ENEJDER & al. [2]).
As an initial evaluation of the ability of UV-VIS spectrophotometry to measure Glu concentration, a series of spectra were collected on human volunteers, in conjunction with a fertility estimate and sperm quality evaluation test. It is known that the human spermatozoon relies on glycolysis as the primary ATP source (K. MIKI & al. [3], C. MUKAI & al. [4]) and the main glycolysis bioenergetical substrate is Glu. Glu, fructose, or mannose is the only sugars glycolysable by human sperm. No significant concentration of mannose is present in human sperm (D. H. OWEN & al. [5]). However many proteins and peptides are present in semen, and they can all absorb in the UV spectral region. Glu has a specific UV absorption peak at 267 nm wavelength (N. D. YORDANOV & al. [6], H. KUBOTA & al. [7]).
UV-VIS spectrophotometry specific spectral features are characteristic for each semen analyte, in contrast with Raman spectroscopy where the sharp spectral features are characteristic for each molecule (M. K. A. ENEJDER & al. [2]).

We tried to determine the Glu concentration in human semen, using the world wide accepted methods for Glu assessment in human fluids: enzymatic colorimetric (PAP), and glucose oxidase (GO), as control.

Determination of Glu can be done using the **PAP method**, according to the following reactions:

$$Glu + O_2 \xrightarrow{\text{Glu oxidase}} \text{Gluconic acid} + H_2O_2$$

$$2H_2O_2 + \text{Phenol} + \text{4-Aminoantipyrine} \xrightarrow{\text{Peroxidase}} \text{Red quinone} + 4H_20$$

The optical density (OD) can be measured after a 10 minute incubation. The measure can be done during 30 minutes for the standard concentration c of mg/100mL, using the calculation formula:

$$C = \text{OD sample x } 100 / \text{OD standard}$$

GO method is based on the reactions:

$$Glu + O_2 \xrightarrow{\text{Glu oxidase}} \text{Gluconic acid} + H_2O_2$$

$$H_2O_2 \xrightarrow{\text{Peroxidase}} H_2O + O_2$$

$$O_2 + \text{Chromogenic } O_2 \text{ acceptor} \longrightarrow \text{Colored complex}$$

The chromogenic oxygen acceptor picks up the hydrogen peroxide produced, and form a color which is proportional to the Glu concentration present in the semen sample.

Optical methods for direct Glu detection have been explored, of which UV-VIS spectrophotometry show promise. A nondestructive measurement of concentration would be particularly beneficial where the results are needed quickly, or where measurements must be taken frequently. An obvious example of this is, in the case of human semen, the measurement of glucose concentration. Since germ cells from human specimens can be used for different reasons (in vitro fertilization, etc.), a cell noninvasive method of measurement would be particularly beneficial in the cases where the samples are rare.

Glu is the main bioenergetical substrate for ATP production via glycolysis, and ATP is the main bioenergetical fuel used by spermatozoa for movement and metabolism (K. MIKI & al. [3], C. MUKAI & al. [4]). As a result Glu concentration in human semen is decreasing in time. Our goal was to determine the average Glu concentration, in human semen of normozoospermic quality. Total semen Glu concentration includes the seminal liquid Glu, and the spermatozoa metabolic inhaled Glu at a time point.

The sample number of spermatozoa cells was estimated using the formula:

$$\text{No. of cells} = NxDFx10^6$$

N = Number of cells counted
DF= Dilution factor

Counting was done before dilution for each and every sample analyzed. As a result for counting we used DF =1 in the above formula. The principle of cells counting using a Makler chamber is based on counting the cells in a 10^{-3} mm^3 well mixed volume of semen (10 squares), and multiplying the result with 10^6 to estimate the number of cells per mL ($1cm^3$). Rapid progressive (RP), slow progressive (SP), and immobile (I) cells concentration were determined after counting by simple math operations. The proportion of RP cells is determined by dividing the number of RP cells counted (cells with a velocity greater than 25 μm/s) in the total number of cells counted, for each and every semen sample in part. SP cells and I cells proportion is determined similar.

Computer assisted semen analysis (CASA) was used for spermatozoa velocity assessment.

For Glu UV peak (GUVP) identification from UV-VIS spectra of human semen we employed three statistical tests: Independent t-Test, One-way ANOVA, Multiple Regression. With two samples of data, one X1 and one X2 column, that are independent and that follow a normal distribution with constant variance, a two-population t-Test, One-way ANOVA and Multiple Regression tests can be employed to test whether or not the population means are the same. In our case the two populations considered are the numerical values of 1: 5 diluted pure Glu UV spectrum (group 1), and the numerical values of the UV spectrum extracted from UV-VIS spectrum of 1:5 diluted human semen (group 2), between 255 nm and 275 nm.

The "Prob > F" column gives the probability of obtaining an F statistic larger than the one listed for the regression, by pure chance. Typically, one wants this number to be 0.05 or less, this is the de facto standard. The R-square statistic measures the amount of the variability in the data that is explained by the linear model. It varies from 0 to 1.

In the present study, a new univariate calibration method, based on 267nm GUVP, was developed for Glu concentration assessment in human semen.

Materials and methods

Our semen samples were donated from the In Vitro Fertilization (IVF) Laboratory (Cluj-Napoca, Romania) according to World Health Organization (WORLD HEALTH ORGANIZATION [8]) criteria, after three days of sexual abstinence. Samples were obtained from a group of 30 donors known to be healthy, white males between the ages of 25 and 39, and with semen samples known to be with normozoospermia. Samples collection was always done on the IVF Laboratory premises by ejaculation.

UV-VIS spectra, each measured for 2 minutes, and reference glucose concentrations (GO and PAP methods) from semen samples were measured as soon as the liquefaction was completed (15-20 min after ejaculation). Each donated specimen was counted and marked in vitro at 22 ± 0.3^{0}C.

For spectrophotometric measurements we extracted quantities of 50 μL (10 times 5 μL) at a time from the main specimen

The well known cells count notations of motility percentage were used to characterize the velocity cells distribution (rapid progressive, slow progressive, immobile %) for all samples. The counting was done by two biologists using the Makler sperm counting method (A. MAKLER [9]) taking independent counts, on 10 Makler counting chamber squares. The counting was repeated 5 times in different locations of the one and same semen sample at a time point

The Makler chamber (Sefi Medical Instruments, Haifa, Israel) was used according to the manufacturer instruction. For best results well-mixed semen (5 μL at a time point) was transferred to the Makler chamber (K. YANAGIDA & al. [10]). Cover was applied promptly, since a delay has been shown to be a potential source of error that results in a higher sperm concentration (P. MATSON & al. [11]). Applying the cover, care was taken to avoid the formation of bubbles.

We used a UV-VIS JASCO 530 double beam spectrophotometer (Jasco UV/Vis Gross-Umstadt, Germany), single monochromatic, and with silicon photodiode detector. The spectrophotometer was set for Abs mode, has 0.5 nm wavelength accuracy, and works based on the principles of absorption spectra.

The best dilution concentration, for GUVP identification, appears to be between 20%, 1:5 respectively. In this dilution interval the GUVP can be easy identified and used. Type I, 18 MΩ deionized distillated water was used for preparation of all diluted samples.

We extracted and identified the Glu specific UV-VIS spectrum, using water diluted pharmaceutical purity Glu powder (99%, UMF, Cluj-Napoca, Romania).

Pharmaceutical purified Glu powder was weighted for calibration, using a KERN ABJ scale, with a 0.1 mg error, and we added distillated water to obtain 100 mL. Successive concentration samples were prepared, containing between 10.1±1mg/100mL and 140.1±1mg/100mL Glu concentration, using the KERN ABJ scale, and several marked pipettes. Each diluted sample was mixed and allowed to equilibrate 2–3 min. The mixing was repeated three times for each concentration. UV-VIS spectra were drawn using 0.5 mL and 2 mm thickness cuvees for semen sample and reference.

We identify GUVP from each and every concentration samples prepared. According to the UV spectra, water solutions show well pronounced absorption bands, with a peak at 267 nm for Glu (M. K. A. ENEJDER & al. [2], K. MIKI & al. [3], N. D. YORDANOV & al. [6]). We did not use any chemical reagents for our method, based on GUVP extraction, and as a result no chemical specific interaction of Glu was necessary to chemically identify Glu.

At 267 nm one can expect the mixed UV-VIS absorbance of all molecules present in human semen, which constitutes the background of the semen sample. The whole idea of this work is based on the extraction of the GUVP, at 267 nm, from the UV-VIS spectra of 1:5 diluted semen samples. Using 3rd grade regression techniques (Origin 6.1) we extracted the UV-VIS spectral background, for each and every semen sample considered. The principle of extracting Glu UV spectrum from each and every UV-VIS semen sample is very simple: subtract analysis between the UV-VIS graph of the semen sample, and the UV-VIS graph of the spectral background of the same semen sample.

The UV absorbance values increment from all other semen components, at 267 nm, was estimated and eliminated for each and every semen sample in part.

For PAP and GO control methods we used a Konelab 30 I (Thermo - Finland), with reagents from Diagnosticum Zrt. (Hungary). We used the reagents R, R1 and R2 by the manufacturer instructions. The PAP necessary reagent R has in composition:

Phosphate buffer	13.8 mmol/L	(pH 7.40)
Phenol	10 mmol/L	
4-Aminoantipyrine	0.3 mmol/L	
Glu oxidase	≥ 10 000	U/L
Peroxidase	≥ 700	U/L

This reagent can be used manually with a standard sample of 10 µL, at wavelength 500 nm (492-550), and on most analyzers.

For GO method we used a simple manual procedure, which has reported results for the same type of human fluid (P. IGHINADUWA & al. [12]).

A statistical analysis of repeatability was also performed. The repeat results were analyzed using SPSS 13.0 for Windows (SPSS Inc, Chicago, Ill). Makler undiluted and Makler 1:1counts were tested systematically for absolute agreement using Intraclass Correlation Coefficients (ICC) and the 2-way mixed effects model. Coefficients of Variation (CV) were also calculated, to determine the spread of the repeat counts relative to the mean for each method. The 95% confidence intervals (CI) were also measured for each method. Glucose concentration results presented are significant at the $p<0.05$ level.

Results and discussions

We extracted the UV-VIS spectra from 1:5 water diluted solutions from all major biochemical components of healthy human semen (C. MUKAI & al. [4]). Concentrations were respected as proposed (C. MUKAI & al. [4]) (basic results graphs not presented). No other semen component has a clear UV absorption band with a peak at 267nm, except for Glu

(Fig. 1a). To avoid unwanted errors we extracted the UV-VIS spectrum of the cuvee filled with water (Fig. 1b). We subtracted the water spectrum from all the spectra we analyzed, using analysis from Origin 6.1 (basic results graphs not presented).

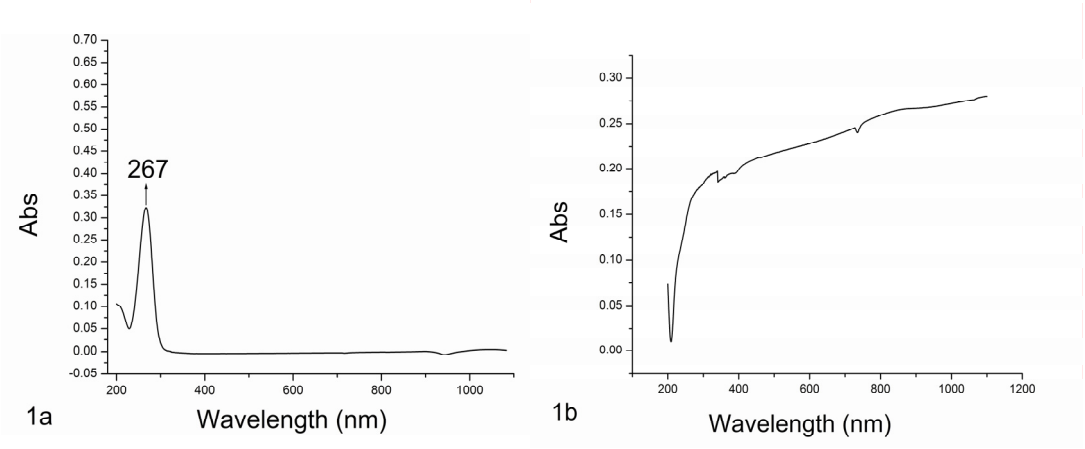

Figure 1. UV-VIS spectrum of 1:5 diluted Glu solution, for 55 ±1 mg/100mL glucose concentration (a). UV-VIS spectrum of the cuvee used filled with water (b).

According to the UV-VIS spectra, 1:5 diluted human semen show a UV well pronounced absorption band with a clear peak at 267 nm (Figure 2a).

From basic analysis (Origin 6.1) the semen spectral background resulted can be fitted using a 3rd grade polynomial regression (Fig. 2b).

The corresponding equation (1) for the area of interest (between 230 and 300nm), which best approximates the semen spectral background is:

$$Y = 452.71657 - 5.17716\ X + 0.01979\ X^2 - 2.51964E\text{-}5\ X^3 \quad (1)$$

In equation (1) Y represents the UV-VIS Abs of the spectral background, of a (72, 0, 28 %) human semen, and X the corresponding wavelength. The regression parameters are summarized (Tab. 1).

From (1) and (Fig. 2a) the 267 nm GUVP can be extracted using simple Origin 6.1 analysis (Fig. 2b). To ensure that the peak we measure in the human semen, it is indeed the GUVP, we compare and analyzed the 1:5 water diluted Glu peak (Fig.1a), with the related peak in the 1:5 diluted semen spectrum (Fig. 2a).

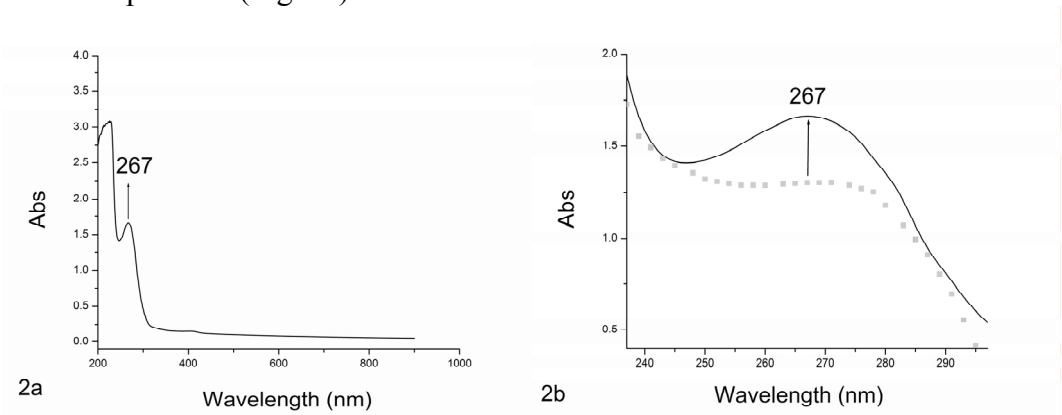

Figure 2. UV-VIS spectrum (black) from a healthy (72, 0, 28 %) 1:5 diluted human semen (a) and 3rd grade polynomial regression for the spectral background (dotted gray) (b).

We used, Independent t-Test, One-way ANOVA and Multiple Regression for comparison (Table 2).

Table 2. Comparison between Glu specific UV absorption peak, extracted from human semen (72, 0, 28 %), and pure Glu specific UV reference peak at 267 nm

Parameter / Method	Independent t-Test	One-way ANOVA	Multiple Regression
p	0.27754	0.27754	0.027754
t	-1.10166	-	-
F	-	1.21364	1.21364
R^2 (COD)	-	-	0.90335
Prob>F	<0.0001	<0.0001	<0.0001
Final test conclusion	same means	same means	same means

In order to obtain a calibration graph of the water diluted Glu, the absorbance was measured at 267 nm (Fig.3a).

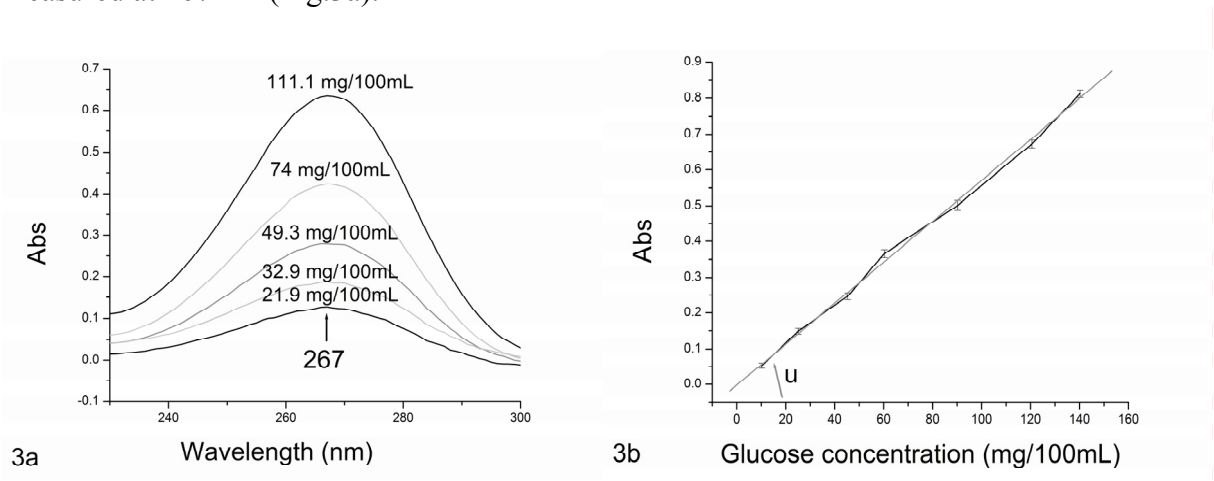

Figure 3. UV spectra of 1:5 diluted glucose (a).Linear fit of Glu concentration scale resulting, plotting UV-VIS absorbance, against concentration of 1:5 diluted Glu (b), for 267 nm.

A calibration graph plotting absorbance against concentration of Glu, in the range 10±1mg/100mL to 140±1mg/100mL was determined and linear fitted by polynomial regression (Fig.3b).The characteristics of the calibration graph and the statistical parameters for determination of Glu under optimum conditions are summarized.

The concentration calibration scale resulted (Fig.3b) was fitted linear by polynomial regression with the corresponding equation:

$$y = (5.72111\ E\text{-}3)\ x + 3.6226E\text{-}5 \quad (2)$$

in which x represents the value of Glu sample concentration, and y is the absorbance value at 267 nm for the same sample.

In equation no (2), first coefficient error is ± 0.02413 E-3.

For the polynomial regression of the Glu UV concentration for the scale calibration (Fig.3b), the coefficient of determination (COD) value is R^2=0.99864. This concentration scale calibration (Fig.3b) is significant at $p < 0.05$ level.

From (Fig.3b) considering the second term in equation no (2) as insignificant, results:

$$x = y \operatorname{ctg} u \quad (3)$$

In (2) u is the angle between the linear fit of the calibration graph of Glu concentration, and OX axe (Fig.3b). Equation (2) applies to 1:5 water diluted Glu solution. From equations (2) and (3) we have:

$$\operatorname{ctg} u = 174.7915 \quad (4)$$

From (4) and (3), we can determine the total Glu concentration in human semen x, as a function of the absorbance values y of the GUVP, at 267nm (5):

$$x = 174.7915\ y \quad (5)$$

Equation (5) is significant at $p<0.05$ level, and the results for the total Glu concentration in human semen x, are in the usual units of mg/100mL.

We used GUVP method to determine the total human semen Glu concentration for 30 healthy donors, known to be very fertile. The results revealed that the average Glu content in normozoospermic human semen, is 47.17±4.13 mg/100mL ($p < 0.05$, $R^2 = 0.9801$) (Tab. 3).

Table 3. Resulting Glu concentration (column 4, 8) of human (RP, SP, I) semen type, for 30 samples (#). Glu 267 nm UV specific peak Abs values shown (column 3, 7)

#	RP, SP, I (%)	Glu UV peak (Abs value ± SD)	Glu concentration (mg/100 mL)	#	RP, SP, I (%)	Glu UV peak (Abs value ± SD)	Glu concentration (mg/100 mL)
1	72, 0, 28	0.32832±0.01429	57.3±2.7	16	57, 14, 29	0.27914±0.01238	48.8±2.4
2	60, 9, 31	0.25339±0.01147	44.2±2.3	17	56, 20, 24	0.27171±0.01326	47.5±2.7
3	61, 10, 29	0.25911±0.01236	45.2±2.4	18	57, 18, 25	0.24941±0.01163	43.6±2.3
4	54, 22, 24	0.26712±0.01161	46.6±2.3	19	50, 21, 29	0.24253±0.01369	42.4±2.7
5	59, 11, 30	0.23795±0.01212	41.5±2.4	20	59, 6, 35	0.29574±0.01146	51.7±2.3
6	63, 21, 16	0.27742±0.01326	48.5±2.6	21	58, 11, 31	0.28201±0.01451	49.3±2.8
7	64, 15, 21	0.27227±0.01291	47.5±2.5	22	64, 26, 10	0.27229±0.01254	47.6±2.4
8	52, 27, 21	0.25968±0.01257	45.3±2.4	23	69, 8, 23	0.27286±0.01362	47.7±2.8
9	57,16,27	0.23966±0.01269	41.8±2.5	24	67, 2, 31	0.29288±0.01268	51.2±2.5
10	59, 11, 30	0.28771±0.01007	50.2±2	25	63, 14, 23	0.26313±0.01436	46±2.8
11	70, 5, 25	0.30831±0.01402	53.9±2.7	26	54, 11, 35	0.25284±0.01166	44.2±2.3
12	66, 8, 26	0.27914±0.01345	48.8±2.7	27	51, 27, 22	0.23969±0.01364	41.9±2.6
13	71, 9, 20	0.32147±0.01421	56.2±2.7	28	69, 7, 24	0.30032±0.01329	52.5±2.6
14	60, 13, 27	0.23796±0.01367	41.6±2.7	29	62, 6, 32	0.26944±0.01037	47.1±2.1
15	53, 29, 18	0.26027±0.01215	45.5±2.4	30	51, 17, 32	0.22657±0.01114	39.6±2.2

We determined the samples Glu concentration using PAP, GO methods, and our proposed GUVP method (Tab. 4).

Table 4. Average glucose concentration for 30 samples (#), of human normozoospermic (RP, SP, I) semen type, determined using PAP, GO and GUVP methods

#	RP, SP, I %	PAP mg/100 ml	GO mg/100 ml	GUVP mg/100 ml	#	RP, SP, I %	PAP mg/100 ml	GO mg/100 ml	GUVP mg/100 ml
1	72, 0, 28	neg. bias	43.1±6.8	57.3±2.7	16	57, 14, 29	neg. bias	44.5±8.5	48.8±2.4
2	60, 9, 31	neg. bias	40±5.3	44.2±2.3	17	56, 20, 24	0	39.2±9.3	47.5±2.7
3	61, 10, 9	0	38.6±6.2	45.2±2.4	18	57, 18, 25	neg. bias	40.3±7.4	43.6±2.3
4	54, 22, 4	neg. bias	38.3±4.7	46.6±2.3	19	50, 21, 29	neg. bias	41.8±7.7	42.4±2.7
5	59, 11,30	neg. bias	34±3.9	41.5±2.4	20	59, 6, 35	neg. bias	43±7.9	51.7±2.3
6	63, 21, 6	neg. bias	42.9±8.3	48.5±2.6	21	58, 11, 31	0	46.7±8.3	49.3±2.8
7	64, 15,21	neg. bias	43.1±7.9	47.5±2.5	22	64, 26, 10	neg. bias	43.8±5.8	47.6±2.4
8	52, 27,21	0	40.6±9.5	45.3±2.4	23	69, 8, 23	neg. bias	44.5±6.7	47.7±2.8
9	57,16,27	0	36.3±8.4	41.8±2.5	24	67, 2, 31	neg. bias	42±4.9	51.2±2.5
10	59, 11,30	neg. bias	46.4±6.2	50.2±2	25	63, 14, 23	neg. bias	41.3±10.4	46±2.8
11	70, 5, 25	neg. bias	46.1±7.4	53.9±2.7	26	54, 11, 35	neg. bias	41.2±7.3	44.2±2.3
12	66, 8, 26	neg. bias	44.9±8.2	48.8±2.7	27	51, 27, 22	neg. bias	40±9.6	41.9±2.6
13	71, 9, 20	neg. bias	49±8.6	56.2±2.7	28	69, 7, 24	neg. bias	43.8±7.2	52.5±2.6
14	60, 13, 7	neg. bias	38.4±6.9	41.6±2.7	29	62, 6, 32	neg. bias	43.5±8.8	47.1±2.1
15	53, 29,18	neg. bias	41.6±5.7	45.5±2.4	30	51, 17, 32	neg. bias	33.9±10.9	39.6±2.2

The GUVP can be clear separated and identified, from human semen UV-VIS spectra, for 1:5 semen dilution in water (Fig. 2a, 2b). This necessary condition for a univariate

calibration method can be done for our proposed method with se = ±2.39E-4. Other studies report the same results, that Glu has an UV absorption band with a peak at 267 nm (N. D. YORDANOV & al. [6], H. KUBOTA & al. [7]).

All the rest of normozoospermic semen samples have similar behavior, and from all the semen samples, GUVP at 267 nm wavelength, can be extracted using Origin 6.1 (Table 3).

Neglecting the second term in equation (2), gives the equation a physical meaning: for zero Glu concentration, we have zero Glu UV absorption, at 267 nm wavelength. The error associated with this parameter neglecting is 0.00633 mg/100mL, which is insignificant.

Using Konelab 30 I, with reagents from Diagnosticum Zrt., PAP reagents method, cannot be used to determine the concentration of Glu in human semen (Tab.4).

For our assay the lower limit of detection is 5 mg/100mL, which is comparable with all lower limits of detection, for all chemical reagents methods, worldwide accepted, in other human fluids. However, to measure Glu in human semen, using UV-VIS spectrophotometry, is more complex than appear for various reasons: a) the spectra from typical physiological concentrations of Glu in human semen are in the order of ten times lower than the total spectrum from human semen; b) the dilutions have to be the same with the concentration used for concentration scale calibration; c) the other components total spectra background for undiluted samples is stronger than the signal, as a result dilution is necessary; d) although UV-VIS spectra from all other biomolecules that are present in the human semen, do not have clear UV peaks within 19 nm wavelength from GUVP at 267nm, the spectral background overlap to some degree, and therefore interfere. GUVP Abs has to be extracted for each and every sample in part; e) the optical properties of the seminal liquid, as well as the probe depth influence the signal; f) the Glu specific UV chemical shifting is not the same for human semen from different donors, and depends of dilution used; g) the spectrophotometer used has to have thin cuvees, and each absorption measurement has to be as short as possible to avoid unwanted light scattering errors.

Our results suggest that there is a correlation between the Glu content of nomozoospermic human semen, and sperm motility (Table 3). Normozoospermic human semen with greater Glu concentration, have greater RP cells proportion.

Glu assessments in human semen have been performed over the past 100 years, but the reported results are rare and very different, from 39.50 mg/100mL (P. IGHINADUWA & al. [12]) to 71 mg/100mL (D. MONTAGNON & al. [13]). Other authors reports show 25.8 mg/100mL (L. TOMASZEWSKI & al. [14), or even less, 18.3 mg/100mL (I.H. HIRSCH & al. [15]). A human seminal liquid simulant, which contain 102 mg/100mL, have been proposed in 2005 (C. MUKAI & al. [4]). We excluded from our comparative analysis all results dated before 1980 as unreliable data.

The proposed chemical concentration nondestructive method GUVP, was found to be as sensitive as the existing chemical reagents based method GO.

The difference of the average Glu concentration results using GUVP (47.17±4.13 mg/100mL) and GO (41.76±7.79 mg/100mL) methods (Tab.4), would suggest that there is a small, but significant quantity of metabolic inhaled Glu, in normal human spermatozoa after liquefaction (5 to 15 mg/100mL).

Male-factor infertility is involved in fully one-half clinically infertile cases, of approximately 15% of couples attempting to conceive today (C. MUKAI & al. [4]). How the Glu is a major bioenergetical substrate for the human spermatozoa motility (K. MIKI & al. [3], Z. TRUTA & al. [16]) this proposed method for assessment of Glu in human semen, is potentially important.

Conclusions

Using GUVP method, studies are allowed without any chemical interference from the reaction medium, and without need for expensive instrumentation. The new GUVP developed method, has the major advantage that can be used for the assessment of average Glu concentration in human semen, without any necessary substrate or reagents.

Seminal glucose level is an important bioenergetical semen parameter, and is thus recommended as an adjunct to other established biochemical and bioenergetical parameters, for the investigation of spermatozoa metabolism.

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