นิพนธ์ต้นฉบับ

The correlation of fructose : glucose ratio in seminal plasma and spermatozoa motility in fertile and infertile males.

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Levels of fructose and glucose were determined in 226 seminal plasma of Thai men. Of these, 46 were fertile men and 180 were infertile men. The mean concentration of fructose, glucose and the fructose : glucose ratio in seminal plasma of fertile men were 19.31, 0.09 m mol/L and 232.96, and infertile men were 15.85, 0.16 m mol/L and 147.50 respectively. Significant differences in fructose, glucose and fructose : glucose ratios were found between fertile and infertile men ($p < 0.01$). The fructose : glucose ratio indicated a correlation with motility of the spermatozoa ($r = 0.46$, $p < 0.01$). This report will be helpful for the physician in evaluating male infertility.

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ยุวสาร นิติชัย, สมัย ลีพิพัฒน์ไพบูลย์, เย็นจิต จันทรประสิทธิ์,ประมวล วีรุตมเสน, วิสุทธิ์ บุญเกษมสันติ. ความสัมพันธ์ระหว่างอัตราส่วนฟรุคโตสต่อกลูโคสในน้ำอสุจิกับการเคลื่อนที่ของตัวอสุจิ. จุฬาลงกรณ์เวชสาร 2534 กุมภาพันธ์; 35 (2): 91-98

ได้ทำการวิเคราะห์ระดับฟรุคโตสและกลูโคสในน้ำอสุจิของชายไทยจำนวน 226 ตัวอย่าง จากจำนวนตัวอย่างนี้เป็นผู้มีบุตร 46 ตัวอย่างและมีบุตรยาก 180 ตัวอย่าง ผลการวิเคราะห์น้ำตาลฟรุคโตส กลูโคส และอัตราส่วนฟรุคโตสต่อกลูโคสในน้ำอสุจิของกลุ่มมีบุตรได้ค่าเฉลี่ย 19.31, 0.09 m mol/L และ 232.96 ในกลุ่มมีบุตรยากได้ค่าเฉลี่ย 15.85, 0.16 m mol/L และ 147.50 ตามลำดับ ผลวิเคราะห์ของทั้งสองกลุ่มแตกต่างกันอย่างมีนัยสำคัญทางสถิติ ($p < 0.01$) และพบว่าอัตราส่วนฟรุคโตสต่อกลูโคสมีความสัมพันธ์กับการเคลื่อนที่ของตัวอสุจิอย่างมีนัยสำคัญทางสถิติด้วย ($r = 0.46, p < 0.01$) ผลการวิเคราะห์นี้น่าจะเป็นประโยชน์ต่อแพทย์ในการประเมินความผิดปกติทางฝ่ายชาย และอาจนำไปประยุกต์ใช้ในการแก้ไขปัญหาการมีบุตรยาก

Infertility is a major problem in marriages. The medical profession is still trying to find out the causes of this problem. In the case of possible male infertility, semen analysis and analysing the fructose contents are generaly carried out. According to Nagaie et al's report, the spermatozoa with high motility had a better chance for fertilization and the resulting pregnancy.[1] Fructose and glucose are generally acknowledged as being the major energy sources for spermatozoa, fructose concentration is 50 times more concentrated than glucose.[2,3] A previous study demonstrated that hexokinase from human sperm has a Km value in relation to fructose and glucose of 2.82 mM and 0.1 mM respectively. It is evident that glucose is metabolized more readily than frutose in sperm.[4] It seems that glucose is more readily used as an energy source for the motility of spermatozoa than fructose. Legg et al has shown that fructose : glucose ratio is associated with a corresponding decrease in spermatozoa motility.[5] We felt it worth while to compare the ratio of fructose : glucose in Thai men, with the motility of their spermatozoa. The study will provide reference values for evaluating semen quality in infertile cases.

Materials and Methods

The recruitment of volunteers was carried out at the antenatal and infertility clinic of Chulalongkorn Hospital. The volunteers were healthy men whose wives were 3-4 months along in their pregnancy, 46 cases were selected as the control group of fertile men and 180 cases of infertile men. All of the men were between 22 and 52 years old (mean $\pm$ SD = 33.95 $\pm$ 5.90). They did not take any drugs before masturbation and had had 2-7 days of abstinence. Semen was divided into 2 groups : normal motility (motility $\geq$ 50%) and abnormal motility (motility) $<$ 50%). The semen was classified into 3 groups of motility grading :

a) if the spermatozoa has a rapid and linear progressive motility or good progression (grade 3)

b) if it has a slow or sluggish linear or non-linear movement or moderate progression (grade 2)

c) if it has a non-progressive motility (grade 1)

The semen was left at room temperature for 30 minutes to allow liquification to occur, and then the semen was analysed for general appearance, volume, sperm density, motility, grading of motility[6] and viability.[7] The seminal plasma was centrifuged 2000 g at 4°C for 15 minutes. Then seminal glucose and fructose were estimated colorimetrically by a modified method described by Eliasson[8], Trinder[9], Karvonen and Malm.[10]

The standardization of both glucose and fructose were performed by measuring the pool specimens for each batch of reagents. The precision and accuracy of the assay were calculated afterwards. Statistical analysis of the results were performed by using a t-test for unpaired data and a linear regression line.

Results

Figures 1 and 2 show the straight lines between the optical density and the concentration of standard glucose and fructose. The coefficient of variation within batch and between batches of assay was 0.99 and 1.99% for glucose, and 2.18 and 3.37% for fructose (Table 1)

Table 1. Precision and accuracy of assay.

Pool semen	Precision of assay				Accuracy of assay	
	Within assay (n=10)		Between assay (n=26)		Std. added	%
	$\bar{X}\pm SD$	% CV	$\bar{X}\pm SD$	% CV	(m mol/L)	
Glucose (m mol/L)	0.304±0.003	0.99	0.301±0.006	1.99	0.28 0.56	100.3 94.4
Fructose (m mol/L)	20.20±0.44	2.18	19.60±0.66	3.37	2.8 5.6	100.0 101.2

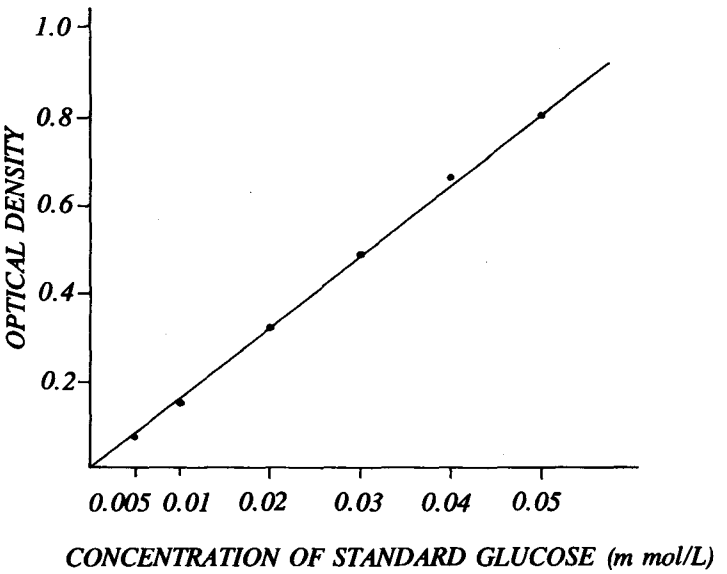

Figure 1. STANDARD CURVE OF GLUCOSE.

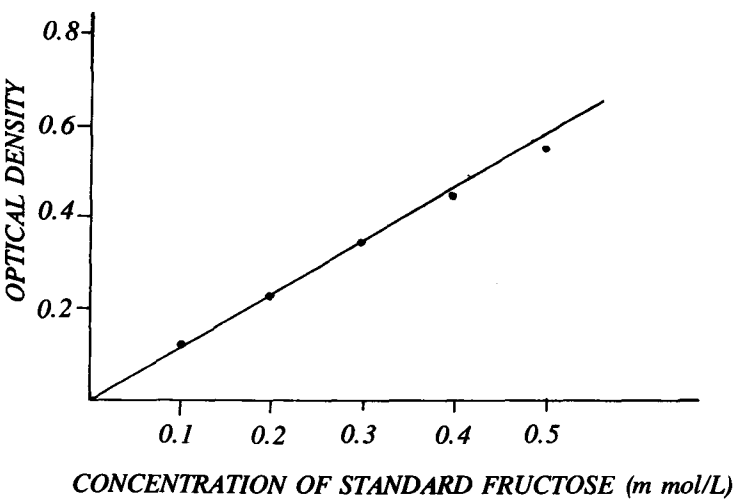

Figure 2. STANDARD CURVE OF FRUCTOSE.

Table 2 shows the results obtained from the 2 seminal fluid groups of fertile and infertile men. Both of them had normal sperm density (20×10^6 /ml), total sperm density (50×10^6) and volume (> 2ml)[6] and there was no statistically significant differences between their mean values. But infertile men had lower motility, viability and normal morphology than did the fertile men. The differences were significant ($p < 0.01$ and $p < 0.05$ respectively)

The mean levels of fructose and the fructose : glucose ratio in fertile men were higher, but glucose was lower than that of infertile men. There were significant differences between the means of these two groups ($p < 0.01$, t-test).

Table 2. The quality of semen and mean levels of sugar in seminal plasma of fertile (n = 46) and infertile men (n = 180).

Semen analysis		Fertile men ($\bar{X} \pm SD$)	Infertile men ($\bar{X} \pm SD$)	
Spermdensity	$X10^6$/ml	51.60±46.79	50.51±52.06	NS
Total spermdensity	$X10^6$	129.76±108.71	138.74±132.40	NS
Motility	(%)	56.84±10.81	48.69±13.49	*
Viability	(%)	62.30±10.83	54.80±14.14	*
Normal morphology	(%)	46.02±15.68	38.73±19.42	**
Volume	(ml)	2.82±1.42	3.05±1.42	NS
Fructose	(m mol/L)	19.31±5.99	15.85±5.94	*
glucose	(m mol/L)	0.09±0.03	0.16±0.12	*
F/G ratio		232.96±72.91	147.50±97.57	*

NS = $p>0.05$

* $p<0.01$; * * $p<0.05$

We furthermore established a correlation between fructose : glucose ratios and motility. There was significant correlation between them ($r = 0.46$, $p < 0.01$).

We also did a comparison between the levels of fructose, glucose and fructose : glucose ratio of fertile men with infertile men according to motility (Figure 3,4) and motility grading (Figure 5,6)

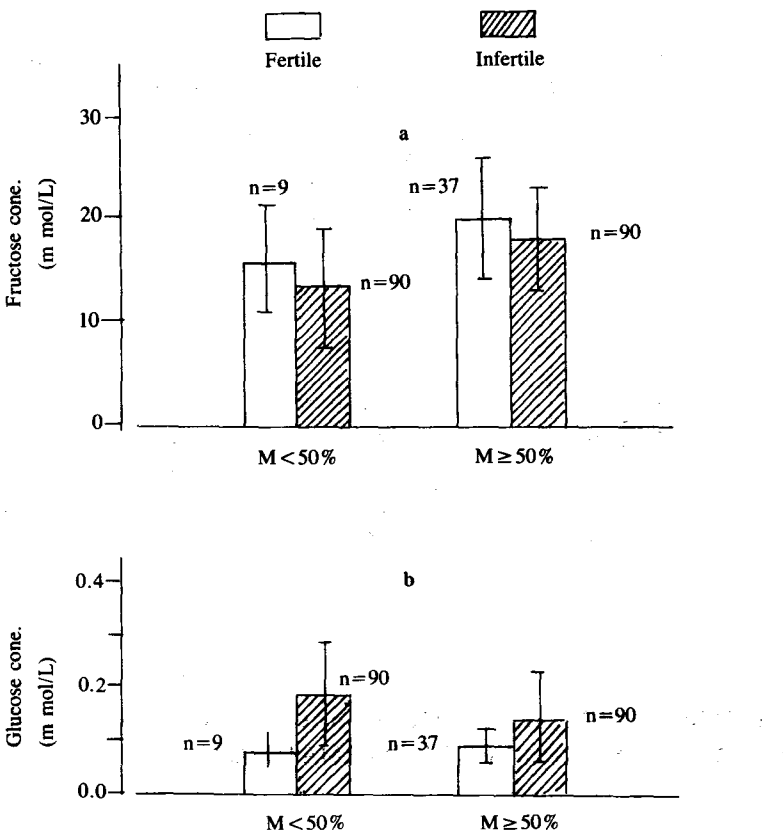

Figure 3. COMPARISON BETWEEN FRUCTOSE (a); GLUCOSE (b) OF FERTILE WITH INFERTILE MEN IN 2 GROUPS OF MOTILITY OF SPERMATOZOA.

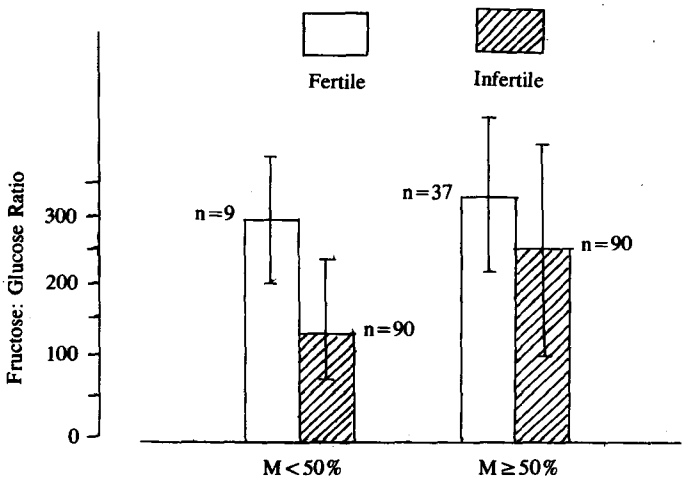

Figure 4. COMPARISON BETWEEN FRUCTOSE : GLUCOSE RATIO OF FERTILE WITH INFERTILE MEN IN 2 GROUPS OF MOTILITY OF SPERMATOZOA.

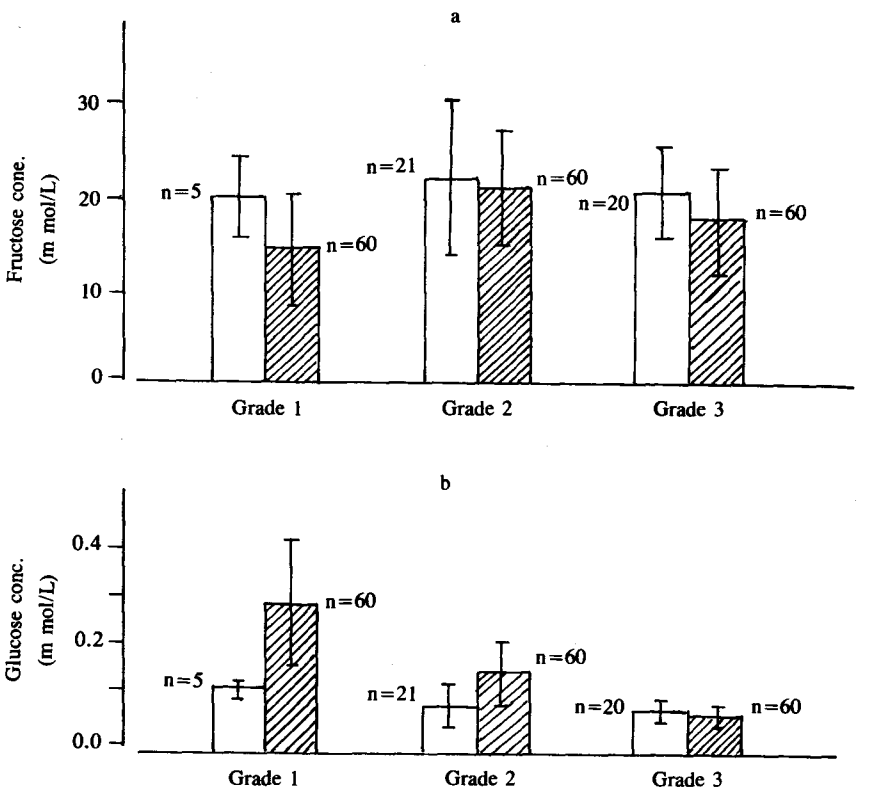

Figure 5. COMPARISON BETWEEN FRUCTOSE (a); GLUCOSE (b) OF FERTILE WITH INFERTILE MEN IN 3 MOTILITY GRADINGS OF SPERMATOZOA.

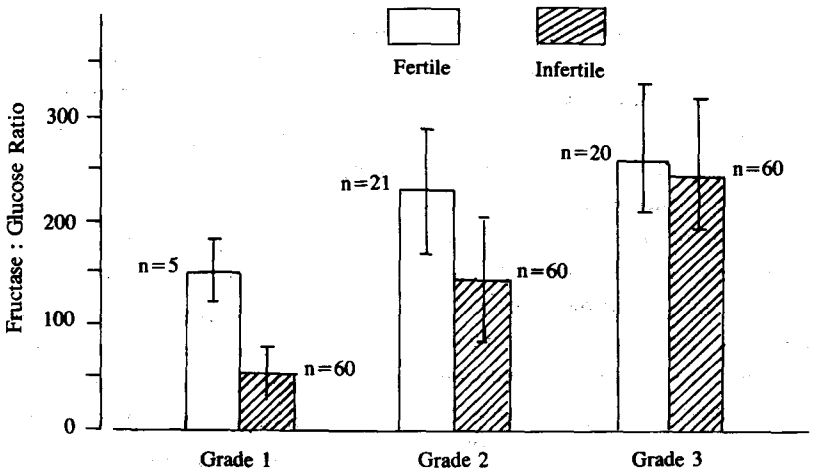

Figure 6. COMPARISON BETWEEN FRUCTOSE : GLUCOSE RATIO OF FERTILE WITH INFERTILE MEN IN 3 MOTILITY GRADINGS OF SPERMATOZOA.

As demonstrated in Figures 3a and 4, the mean levels of fructose and fructose : glucose ratios in fertile men were higher than in infertile men, both for normal and abnormal motility, but the mean level of glucose in fertile men was lower than in infertile men (Figure 3b). There were significant differences in the glucose and fructose : glucose ratios ($p < 0.05$, t-test).

Figures 5a and 6 showed the mean levels of fructose and fructose : glucose ratios in fertile men were higher than infertile men in all motility grading. These differences were statistically significant only in grade 3 for fructose ($p < 0.05$) and grade 1 and 2 for fructose : glucose ratios ($p < 0.001$, t-test)

The mean levels of glucose in fertile men were lower than in infertile men only in grades 1 and 2. There were significant differences between these 2 groups of motility gradings 1 and 2. The mean levels of glucose in grade 3 for fertile men was slighly higher than for infertile men, but it was not a significant difference between them.

Discussion

Glucose and fructose are the major energy sources of movement available to the spermatozoa.[1] Fructose is produced from blood glucose in the seminal vesicles. Glucose utilization appears to be particularly important during the passage of spermatozoa through the cervical mucus.[11] According to Nagai et al's report in 1982, the higher the concentrations of the two sugars (40 mM fructose and 10 mM glucose) the slower the sperm motility. But sperm motility showed maximum activity in the medium added with 0.5 – 1.0 mM glucose or 10 – 20 mM fructose.[1] These findings indicated that both carbohydrates would have optimal concentrations in seminal plasma to facilitate spermatozoa motility. So we tried to find out the appropriate method to evaluate the levels of fructose and glucose in seminal plasma. The methods are simple, accurate and precise (Table 1).

The results of this study indicated that the mean levels of fructose in fertile men was higher than in infertile men (Table 1). Similar findings of Kolkijkovinda et al 1989[13] reported that the mean levels of fructose in infertile men were significantly lower than the normal range. The concentration of fructose in the groups of semen which had normal motility was higher than abnormal motility (Figure 3a). This is similar to Legg et al's report that there was a significantly higher level of fructose in seminal plasma with a normal motility than with an abnormal motility.[5]

Legg et al[5] and Povoa et al[12] have also observed a negative correlation between seminal glucose and sperm motility; a decrease in the level of glucose will increase the motility of spermatozoa.

According to this study, the mean levels of seminal glucose in the infertile men of the abnormal group was statistically higher ($p < 0.05$) than that of the normal motility group (Figure 3b).

However, both fructose and glucose concentration do not indicate the clear movement of spermatozoa. So we calculated the fructose : glucose ratios and found that the higher levels of fructose : glucose ratios were in the good progression group (grade 3), both for fertile and

infertile men (Figure 6). Our results confirm the work of Legg et al[5] but we got a higher ratio. It is probable the different method for measuring the level of sugars accounted for the higher ratio.

We furthermore compared the mean levels of fructose, glucose and fructose : glucose ratios between fertile and infertile men. There was no significant differences between the levels of fructose in fertile and that of infertile men. The only statistical differences were glucose and fructose : glucose ratios which the semen was divided according to the motility of the spermatozoa (Figures 3,4) and motility grading of the spermatozoa (Figures 5,6). There was significant differences only between grade 1 and grade 2. It was possible that, spermatozoa which was in grade 3 had good motility so they were able to make equal use of these two carbohydrates both for fertile and infertile men. The cause of male infertility in grade 3 would have been due to other factors and could be sought out in the future. The results of our study (Figures 3,5) showed that the level of fructose was not as good an indicator as the glucose level and the fructose : glucose ratio for use as a parameter of sperm motility (Figures 4,6). So the levels of glucose and fructose : glucose ratio will be of more benefit to the physicians in the diagnosis of infertile cases and may be used for finding an appropriate way to improve them.

Conclusion

The results of this study indicated that the levels of fructose, glucose and fructose : glucose ratio in seminal plasma of the 46 fertile and 180 infertile men had significant differences between the mean values ($p < 0.01$). The fructose : glucose ratios revealed a correlation with the motility of spermatozoa ($r = 0.46$, $p < 0.01$). The level of fructose : glucose ratio will be more useful in determining a parameter for sperm motility than only glucose or fructose levels.

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