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ORIGINAL ARTICLE

The impact of seminal zinc and fructose concentration on human sperm characteristic

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This study assessed the association between the estimated fructose and zinc concentration and various seminal characteristics. The study participants include 90 male subjects visiting the Komfo Anokye Teaching Hospital between January and July, 2010 for semen analysis as part of routine fertility investigations prior to treatment. Seminal fructose concentration was significantly lower when the normozoospermic group was compared to the oligozoospermic group ($P < .0001$) and in the normozoospermic group compared to the azoospermic group ($P = 0.0096$). A comparison between the oligospermic group and the azoospermic group gave no statistically significant difference. Fructose correlated positively with volume ($r = 0.36$, $P < 0.0001$) and head defect ($r = 0.07$, $P > 0.05$) and negatively with count ($r = -0.21$, $P < 0.05$). Zinc correlated negatively with volume ($r = -0.09$) and head defect ($r = -0.20$) and positively with motility ($r = 0.18$), count ($r = 0.15$) and tail defect ($r = 0.11$). Seminal fructose and zinc concentrations correlated negatively ($r = -0.26$, $P < 0.05$). The role of seminal fructose concentration does not only lie in the assessment of seminal vesicle dysfunction but in conjunction with other seminal properties could give a useful indication of male reproductive function whilst seminal zinc concentration might not be most appropriate for the assessment of male reproductive dysfunction.
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INTRODUCTION

The total number of spermatozoa, the total fluid volume contributed by the various accessory glands, the nature of the spermatozoa (i.e. viability, motility and morphology) and the composition of seminal fluid are important for sperm function (Weiske, 1994; WHO, 2010). They have thus been established as good indicators of human male fertility. An understanding of the factors affecting these characteristics is critical to proper understanding of the mechanisms underlying male infertility (Lewis-Jones *et al.*, 1996; WHO, 2010). Knowledge about the impact of seminal zinc and fructose concentration on sperm characteristics is inconsistent and scanty (Lewis-Jones *et al.*, 1996).

Zinc plays an important role in normal testicular development, spermatogenesis and sperm motility (Lin and Cheng, 1996; Wong *et al.*, 2001). It is a cofactor for a number of metalloenzymes in man. Deficiency of zinc in the reproductive system causes hypogonadism and gonadal hypofunction (Sandstead *et al.*, 1967). Kvist, (1980) and Steven *et al.,* (1982) have reported that zinc in seminal plasma is involved in nuclear chromatin decondensation and acrosin activity. Zinc deficiency in the nucleus may destabilize the quaternary structure of chromatin, a feature important for the fertilizing capacity

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of the spermatozoa. Kvist *et al.,* (1987) and Prasad (1991) conducted an experiment in adult males which revealed that Leydig cell synthesis of testosterone was dependent on adequate dietary zinc. It has also been suggested that zinc is necessary for the conversion of testosterone into its biologically active form 5α-dihydrotestosterone (DHT) via the role of 5α-reductase enzyme (Netter *et al*., 1981) and that depletion of dietary zinc decreases semen volume and serum testosterone levels (Hunt *et al*., 1992). Zinc content in seminal plasma is predominantly secreted by the prostate gland and may reflect prostatic function. However, the association between zinc contents in seminal plasma and other spermatic parameters in both fertile and infertile men is yet to be firmly established.

Apart from zinc, another factor which is essential for spermatozoa metabolism and motility is fructose which serves as an energy source for spermatozoa. It is produced in humans mainly by the seminal vesicles with some contribution from the ampulla of the ductus deferens (Schoenfeld *et al*., 1979). Absence of fructose in patients with low volume ejaculate is indicative of ejaculatory duct obstruction, seminal vesicle dysfunction or hypoplasia (Aumuller and Riva, 1992).

The differences in opinion concerning the concentrations of fructose and zinc in seminal plasma justify further studies into their dynamics *vis a vis* male infertility. The purpose of this study was therefore to determine the association between the estimated fructose and zinc concentration and various seminal characteristics in men referred for semen analysis as part of routine fertility investigations.

MATERIALS AND METHODS

Sample preparation

Zinc and fructose levels in seminal plasma of 90 Ghanaian males (age range 27 – 47 years) referred for semen analysis at Komfo Anokye Teaching Hospital (KATH), Kumasi Ghana, between January and June, 2010, as part of their routine fertility investigations prior to treatment, were used for the study. Semen samples were collected in wide mouthed sterile plastic containers by masturbation after a minimum of 4 days sexual abstinence. Semen analysis (volume, pH, sperm concentration, total count, motility and morphology) of all the samples was performed after liquefaction according to the World Health Organization criteria (1992). On the basis of the assessed parameters, sperm concentration and sperm motility were considered as the important parameters. The study group was assigned into three (3) cohorts based on normal ejaculate (40 million spermatozoa per ejaculate) (normozoospermia), sperm concentration < 20 million mL^{-1} (oligozoospermia) and complete absence of spermatozoa in the ejaculate (azoospermia).

Zinc and fructose assessment

After the semen analysis, samples were centrifuged at 1000 x *g* for 5 min and zinc and fructose concentrations assayed from the supernatant (i.e. seminal plasma). Zinc concentration was assessed using atomic absorption spectrophotometry (Mann, 1964). Fructose content in seminal plasma was determined by the resorcinol method where fructose reacts with resorcinol in concentrated hydrochloric acid (HCl) solution to form a red compound measured at a wavelength of 490 nm against blanks (Mann, 1964).

Statistical analysis

Mean values were compared by the student t-test (unpaired). Categorical variables were compared using the chi-square analysis and correlation determined with the Pearson's correlation coefficient test statistic. All statistical analysis were performed using GraphPad prism version 5.00 for windows (GraphPad software, San Diego California USA, www.graphpad.com).

RESULTS

General seminal properties

The age and general seminal properties of the study population stratified into normozoospermic, oligozoospermic and azoospermic populations are shown in Table 1. Forty one percent (41.1%) of the population were normozoospermic, 51.1% were oligozoospermic and 7.8% azoospermic. No significant difference was observed when the mean liquefaction time in males with normozoospermia (55.50 ± 4.41 min) was compared to males with oligozoospermia (51.52 ± 3.69 min). However, a comparison of the mean liquefaction times in both males with normozoospermia and oligozoospermia to males with azoospermia (19.50 ± 4.50 min) showed a significant difference ($P < 0.001$).

About 45.7% of the males with oligozoospermia had seminal pH less than 7.2 compared to 21.6% of males with normozoospermia ($P < 0.05$). The mean count (60.41 ± 5.72 millions mL^{-1}) and the mean motility (67.08 ± 4.13%) in males with normozoospermia were significantly higher than that in males with oligozoospermia (6.36 ± 0.65 millions mL^{-1} and 31.76 ± 2.71% respectively) ($P < 0.0001$). About 21.7% of males with oligozoospermia had seminal head defects compared to 5.4% in males with normozoospermia ($P < 0.05$) but no significant difference in tail defect was observed when

Table 1 - General characteristics of the study population stratified by count

Variables	Normospermic 37 (41.1%)	Oligospermic 46 (51.1%)	Azoospermic 7 (7.8%)
Age (years)	36.57 ± 1.30	37.46 ± 0.98	36.43 ± 1.81
Abstinence (days)	8.35 ± 1.30	6.67 ± 0.62	13.14 ± 4.72‡‡
Liquefaction (min)	55.50 ± 4.41	51.52 ± 3.69	19.50 ± 4.50##‡‡
Motility (%)	67.08 ± 4.13	31.76 ± 2.71***	0.00 ± 0.00
asthenozoospermia (< 50%)	7 (18.9)	32 (69.6)***	0 (0.0)
normal (≥ 50%)	30 (81.1)	14 (30.4)	0 (0.0)
Volume (ml)	2.99 ± 0.22	3.37 ± 0.24	2.96 ± 0.36
less than 2.0 ml	7 (18.9)	7 (15.2)	1 (14.3)
Count (millions ml^{-1})	60.41 ± 5.72	6.36 ± 0.65***	0.00 ± 0.00
Total count (per ejaculate)	190.60 ± 27.32	20.23 ± 2.62***	0.00 ± 0.00
pH	7.61 ± 0.08	7.53 ± 0.09	7.50 ± 0.27
less than 7.2	8 (21.6)	21 (45.7)*	4 (57.1)
> 7.8	9 (24.3)	15 (32.6)	2 (28.6)
Haematospermia (per HPF)	0	3	0
Pyospermia (per HPF)	5	4	3
Head defect	1 (5.4)	10 (21.7)*	0 (0.0)
Tail defect	35 (94.6)	43 (93.5)	0 (0.0)

Results are presented as mean ± SD. *p < 0.05, **p < 0.001, ***p < 0.0001 defines the level of significance when normospermic was compared to oligospermic (unpaired t-test); #p < 0.05, ##p < 0.001, ###p < 0.0001 defines the level of significance when normospermic was compared to azoospermic (unpaired t-test); ‡p < 0.05, ‡‡p < 0.001, ‡‡‡p < 0.0001 defines the level of significance when oligospermic was compared to azoospermic (unpaired t-test). Categorical variables were compared with Chi-square test statistic.

the proportion in males with normozoospermia was compared to that of males with oligozoospermia.

Zinc concentration and seminal parameters

Even though the mean seminal zinc concentration in males with normozoospermia (101.20 ± 2.24 mg dL^{-1}) was slightly higher than that in males with oligozoospermia (97.75 ± 2.17 mg dL^{-1}) and azoospermia (94.62 ± 6.74 mg dL^{-1}) these differences did not reach a significant level (Figure 1B). Seminal zinc concentration correlated positively with motility (r = 0.18), count (r = 0.15), total count (r = 0.16) and tail defect (r = 0.11) and negatively with head defect (r = -0.20) without any statistical significance (Table 2).

Fructose concentration and seminal parameters

The mean fructose concentration in males with normozoospermia was significantly lower compared to males with oligozoospermia (P < 0.0001) and males with azoospermia (P = 0.0096). There was however no statistically significant difference in the mean fructose concentration in males with oligozoospermia compared to males with azoospermia (Figure 1A). Fructose showed a significant negative correlation (r = -0.21) with count. Negative correlations were also observed with motility (r = -0.04), total count (r = -0.05) and tail defect (r = -0.18) with no statistical significance. A positively non-significant correlation was observed between fructose and head defect (Table 2). Seminal fructose concentration correlated negatively, in a statistically significant way, with zinc concentration (r = -0.26) (Table 2).

DISCUSSION

The WHO criteria for normal semen includes a volume of 2.0 ml or more, sperm concentration of 20 million mL^{-1} or more, sperm motility of 50% or more with forward movement and sperm morphology of 30% or more of normal forms (World Health Organization, 1992). The mean seminal volume of the three study groups classified by count was higher than the documented 2.0 mL and is in agreement with other study

(Promdee and Pongsritasana, 2005). This could be due to the fact that in using at least one abnormal parameter to classify the samples, other parameters e.g. volume could be normal. This study observed positive but not significant correlations between zinc content of seminal plasma and motility, total count and sperm concentration. Stanwell-Smith (Stanwell-Smith *et al.*, 1983), found a positive correlation between sperm concentration and zinc level in fertile men but not in infertile men and (Wong *et al.*, 2000) reported increased proportion of spermatozoa with progressive motility after oral zinc supplementation which corroborates very well with the correlation results observed in this study. The relationship between seminal zinc content and acrosin and nuclear chromatin activity

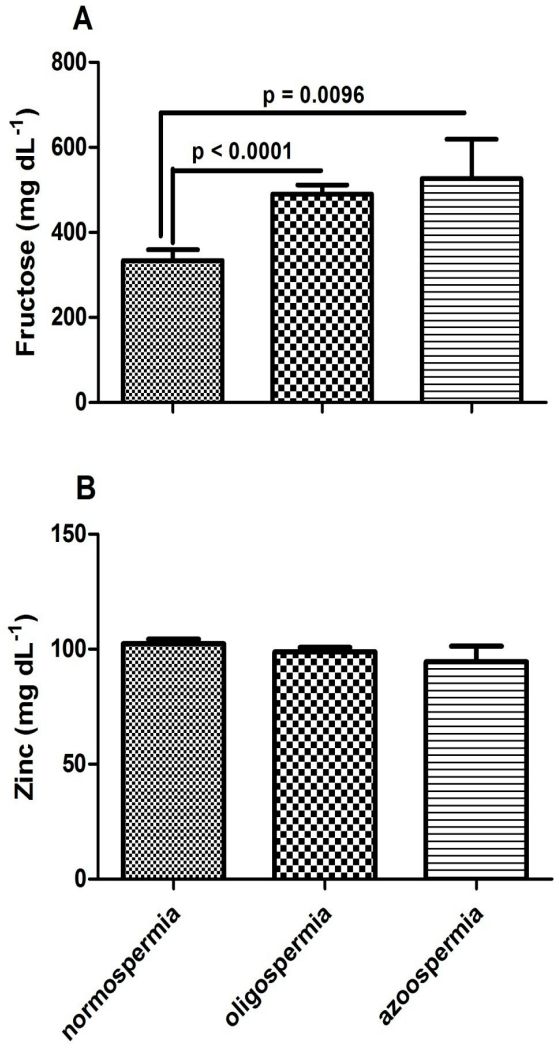

Figure 1 - Seminal fructose (A) and zinc (B) concentrations in the three study categories

Table 2: Pearson product correlation coefficient among the study variables

Variables	AB	Motility	Volume	Count	TC	Zinc	pH	LQ	Immotile	HD	TD	Fructose
Age (years)	0.07	-0.29*	-0.07	-0.06	-0.09	-0.08	0.11	0.05	0.19	0.22*	-0.04	-0.05
AB (days)		-0.20*	0.17	0.08	0.16	0.06	0.07	0.09	-0.07	0.09	-0.23*	0.01
Motility (%)			0.01	0.66***	0.56***	0.18	-0.02	0.05	-0.54***	-0.23*	0.47***	-0.04
Volume (ml)				-0.01	0.27*	-0.09	0.1	-0.03	0.01	0.12	-0.05	0.36***
Count (mil mL-1)					0.89***	0.15	0.06	0.03	-0.41***	-0.12	0.18	-0.21*
TC (ejaculate-1)						0.16	0.12	-0.01	-0.36**	-0.02	0.07	-0.05
Zinc (mg dL-1)							-0.07	0.06	-0.05	-0.2	0.11	-0.26*
pH								0.40***	0	0.11	-0.22*	0.13
LQ (min)									0.05	-0.04	0.03	0.13
Immotile (%)										0.24*	0.18	-0.07
HD											-0.33**	0.07
TD												-0.18

****Correlation is significant at 0.05 level (2-tailed), **Correlation is significant at 0.001 level (2-tailed) and ***Correlation is significant at 0.0001 level (2-tailed). TC = Total count, LQ* = Liquefaction, AB = Abstinence, HD = Head Defect, TD = Tail Defect**

as related in the studies of Kvist (1980) and Steven *et al.,* (1982) was clearly observed in the negative correlation between seminal zinc content and seminal head defect observed in this study. Zinc deficiency might therefore affect acrosin and chromatin activity thereby leading to increased head defects which further reduces the fertilizing capacity of spermatozoa. Wong *et al.,* (2001) demonstrated that zinc content in fertile men were not different from those of infertile men. Abou-Shakra *et al.,* (1989) reported that zinc content in men grouped by sperm concentration was not different from each other. Zinc content in the three study categories grouped by sperm concentration did not show any significant difference although values in normozoospermic males were higher than that in oligozoospermic males which was also higher than that in azoospermic males. This is in agreement with the work of (Wong *et al*., 2001).

Determination of seminal fructose concentration has been used in the 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 vesicles and low seminal fructose concentration. When ejaculatory ducts are blocked, fructose concentration in seminal plasma usually decreases and may become undetectable (Coppens, 1997). Additionally, seminal plasma fructose concentration determination is useful for auxiliary diagnosis of obstructive and non-obstructive azoospermia. Seminal fructose concentration in non-obstructive azoospermia is usually higher than or equal to that in males of normal fertility (Buckett & 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 has also been found in patients with congenital vas deferens-seminal vesicle developmental defect (Kise *et al*., 2000; Kumar *et al*., 2005).

Significantly higher seminal fructose concentrations were observed in azoospermic patients in this study compared to that in oligozoospermic and normozoospermic patients which rules out the possibility of obstructive azoospermia or inflammation of the male accessory glands in azoospermic patients in this study as related to the studies of Buckett and Lewis-Jones, (2002), Manivannan *et al.,* (2005) and Coppens, (1997). This finding confirms an abnormality in the normal function of the seminal vesicles other than obstruction or inflammation which may require further investigation. Montagnon *et al.,* (1990) reported that fructose concentration in seminal plasma is one of the most important markers of seminal vesicular function and that when seminal vesicular function is decreased, semen coagulation, sperm motility, stability of sperm chromatin and semen immune-protection are affected. A definite ratio between the fructose level and the number of spermatozoa in the ejaculate has also been documented; as such, an increase in the number of spermatozoa is generally accompanied by significant fall of fructose in the semen (Biswas *et al*., 1978; Rajalakhshmi *et al*., 1989).

Negative correlations were observed between fructose content and sperm concentration, total count and motility. Fructose concentration has been noted to be essential for spermatozoa metabolism and motility as an energy source (Schoenfeld *et al*., 1979). The reduced fructose concentration in normozoospermic males compared to oligozoospermic males in this study could be attributed directly to the significantly higher mean motility and sperm counts in normozoospermic males which would have used up fructose as an energy source. This finding is in line with that of Lu *et al.,* (2007) who reported a significantly positive correlation between motile sperm concentration and decrease in fructose concentration and further demonstrated that motile sperm in vitro could unceasingly consume fructose. Biswas *et al.,* (1978) and Schirren *et al.,* (1979) also reported decreased fructose concentrations with increasing sperm density and motility. A further finding of a negative correlation between fructose concentration and tail defect further buttresses the need for fructose as an energy source for effective motility of spermatozoa as related in the study of Schoenfeld *et al.,* (1979). Fructose showed a negative correlation with seminal head defect showing that fructose in semen has very little to do with the maintenance of the acrosome and nuclear chromatin activities.

A significant inverse relationship was also observed between zinc and fructose concentrations emphasizing the fact that for effective seminal fluid activity, zinc and fructose concentrations should always be inversely related to each other.

CONCLUSIONS

Seminal zinc plasma levels may not be an appropriate indicator of male reproductive dysfunction and the role of seminal fructose concentration does not only lie in the assessment of seminal vesicle dysfunction but in conjunction with other seminal properties could give a useful indication of male reproductive function.

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