A Cross-sectional Study of the Relationship of Serum Folic Acid, Vitamin B_{12}, Zinc, Magnesium with Semen Parameters in Infertile Couples in Hong Kong

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Objectives: The primary objective was to evaluate the association of serum folate, vitamin B_{12}, zinc, magnesium with semen parameters in infertile couples in Hong Kong. The secondary objective was to explore whether the study participants were deficient in any of these micronutrients. The impact of smoking on semen parameters was also analysed.
Methods: This cross-sectional study recruited 196 Chinese men from the Subfertility Clinic of the Department of Obstetrics and Gynaecology, Kwong Wah Hospital between August 2012 and November 2013. Semen and blood samples were collected on the same day for analysis.
Results: Higher levels of serum magnesium were significantly associated with higher percentages of normal sperm morphology (p=0.02). The median levels of micronutrients studied were within the normal range, indicating that deficiency of these nutrients was rare in our locality, although such deficiency is common in western countries. Both median semen volume and sperm concentration were within the normal range but the median percentages of normal sperm morphology and motility were below the normal range. We postulate that sperm morphology might be improved by an increased intake of magnesium. Smoking was significantly associated with low semen volume (p=0.01).
Conclusion: This is the first study to evaluate the relationship between serum micronutrients and semen parameters in infertile Chinese couples in our locality. Male subfertility is a multifactorial disorder. Although both nutritional and lifestyle factors are important and modifiable, further research on these subjects would provide a platform for potential fertility treatments.
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Introduction

Approximately 16% of couples in Hong Kong are involuntarily childless[1]. This has a great influence on the quality of life[2]. Male infertility contributes to 30% to 50% of all infertility cases[3]. Several animal studies have demonstrated the effects of micronutrients such as folate, vitamin B_{12}, zinc, and magnesium on spermatogenesis[4-7].

Folate is essential for processes that are important for spermatogenesis including DNA synthesis[8], regulation of DNA transcription via methylation[3], as well as transfer RNA and proteins. Vitamin B_{12} is an essential component of DNA synthesis and is also a cofactor in the folate-dependent conversion of homocysteine to methionine. The impact of folate and vitamin B_{12} on semen parameters is controversial. Serum folate and vitamin B_{12} levels have been reported to be lower in infertile subjects[9], although other studies have shown no such association[10,11].

Zinc serves as a cofactor for more than 80 metalloenzymes involved in DNA transcription, expression of steroid receptors and protein synthesis[12-15]. A lowered serum zinc level has been shown to be more common in infertile patients[16]. Zinc is important in testicular development, spermatogenesis, and sperm motility[17]. It

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improves spermatogenesis in animals[15], and increases sperm concentration[18,19], motility[20], and morphology in subfertile males[21].

Magnesium is an essential ion for enzyme activations in the body that are related to male sexual functions[22], including various biochemical processes of spermatogenesis, and functions in sperm motility. Biochemical processes include synthesis of ATP, cAMP, proteins and DNA, ATP hydrolysis, and the functioning of enzymes and non-enzymatic factors involved in proper protective antioxidant mechanisms[23-25]. It is proposed that ATPase releases energy required for sperm motility from ATP[26]. According to Morisawa and Okuno[27], sperm motility requires both cAMP and magnesium ATP, and the formation of them is magnesium-intensive. In-vivo magnesium has been shown to increase sperm motility and sperm production by up to 80%[28], although studies of the impact of serum magnesium on semen variables are scarce.

Most studies have been conducted among a western population. We hypothesised that deficiency in these serum micronutrients would also aggravate semen quality in our Chinese population. The primary objective of our study was to thus evaluate the association of serum folate, vitamin B_{12}, zinc, and magnesium, with semen parameters in infertile couples in Hong Kong. The secondary objective was to explore whether a deficiency of the studied micronutrients was present among the study participants. We also analysed the impact of smoking on semen variables. Although causes of male subfertility are multifactorial, nutrition and lifestyle factors are modifiable. We hoped to gain an invaluable insight into the potential nutrients that improve semen quality, and thus explore the options for fertility treatment.

Methods

This was a cross-sectional study. Chinese couples were recruited when they attended the Subfertility Clinic of the Department of Obstetrics and Gynaecology, Kwong Wah Hospital between August 2012 and November 2013. Infertility was defined as failure to conceive over 1 year of regular intercourse without contraception. Subjects who were non-Chinese and mentally incompetent were excluded. Participants were given written information regarding the objectives and details of the study and informed consent was obtained prior to study recruitment. The sample size was calculated based on the formula:

$$n = \frac{z^2_{1-\frac{\alpha}{2}}\, p(1-p)}{d^2}$$

where α is the significance level (α = 0.05), $z_{1-\frac{\alpha}{2}}$ means the corresponding z value from standard normal distribution (mean = 0, standard deviation = 1), p being the response rate, and d being the margin of error. Assuming p (response rate) to be 80% and d (margin of error) to be 0.06, the required sample size (n) was 171.

Study subjects who attended the Subfertility Clinic were asked to save a semen sample for analysis and blood was taken. One semen sample and blood sample were collected from each subject on the same day. Demographic and clinical data were obtained from the subfertility assessment forms in the electronic patient record through the Clinical Management System of the Hospital Authority, Hong Kong, and included age, occupation, smoking status, history of medical illness or operations, history of genital infection or injury and any coital problem. The study was approved by the Ethics Committee of Kowloon West Cluster, Hospital Authority, Hong Kong.

Semen Analysis

The participant produced a semen sample by masturbation. Self-reported duration of ejaculation abstinence and time of ejaculation were obtained. The whole ejaculate of semen was required to be collected without a condom at home by masturbation into the container provided, then tightly capped. Time of semen collection was recorded; 2 to 3 days of sexual abstinence was advised prior to semen collection. The samples were delivered to the Andrology Laboratory within 1 hour of collection. Once received, the semen was allowed to liquefy for 30 to 60 minutes at room temperature before semen analysis was performed. Semen analysis was performed according to the fifth edition of the World Health Organization (WHO) laboratory manual for the examination and processing of human semen[29]. Motility of sperm was classified as progressive (PR) or non-progressive (NP). Semen analysis was performed in the Andrology Laboratory of Dr Stephen Chow Chun-kay Assisted Reproduction Centre of Kwong Wah Hospital by trained medical technicians. Semen variables were categorised as normal if they were equal or above the reference values, and abnormal if below the reference values according to the WHO 2010's reference values[29].

Blood Measurements

Venous blood samples were drawn into vacutainer tubes and analysed in the Laboratory of Department of Pathology, Kwong Wah Hospital. For serum folate and serum vitamin B_{12}, 4 mL clotted blood was analysed using chemiluminescent microparticle immunoassay on Abbott Architect i2000SR System (Abbott, US). For serum zinc, 4 mL clotted blood was analysed using ICP-AES (inductively coupled plasma atomic emission spectroscopy) on Varian Vista-MPX CCD Simultaneous ICP-AES System (Varian, Australia). For serum magnesium, 4 mL clotted blood was analysed using calmagite spectrophotometry on Beckman Coulter UniCel DxC800 Synchron Clinical System (Beckman Coulter Inc., US). The normal reference ranges of the laboratory of the Department of Pathology of Kwong Wah Hospital for each micronutrient level were as follows: serum folate (3.10-20.50 ng/mL), serum vitamin B_{12} (187-883 pg/mL), serum zinc (10-19 µmol/l), and serum magnesium (0.70-1.05 mmol/l). The serum level of these micronutrients in each blood sample was categorised as deficient if it was below normal range, sufficient if it was within the normal range, and high for those above the normal range.

Statistical Analyses

Analyses were performed using SPSS version 20 for Mac (SPSS Inc., Chicago [IL], US). The association between semen parameters and micronutrient levels was examined as categorical data using exact Chi-square test and Fisher's exact test. A p value of <0.05 was considered statistically significant. Continuous variables were presented as median (interquartile range). The multiple logistic regression analysis (stepwise) was performed to include variables found to be significant at $p<0.2$ by univariate analysis, if considered to be an important demographic variable.

Results

Demographic and Clinical Data

A total of 235 couples were approached during the study period from August 2012 to November 2013. Of the 200 couples recruited, four non-Chinese men were excluded, thus semen and blood samples from 196 Chinese men were analysed. The response rate was 85.1% (200/235).

Table 1[29,30] summarises the demographic and clinical data of the participants as well as their semen variables. The median age of participants (n=196) was 37 years. Demographic details were as follows: 91 (46.4%) worked at administrative levels and as professionals while 26 (13.3%) engaged in labouring work; 122 (62.2%) were non-smokers, 57 (29.1%) were smokers and 17 (8.7%) were ex-smokers. Nine had hypertension, four had diabetes mellitus, three had hyperlipidaemia, two were obese, two had gout, two had hypothyroidism and were prescribed T4 supplementation, 12 had a history of mumps, two had varicocele diagnosed in the male subfertility clinic following referral for azoospermia, and one had previous surgery for varicocele. For history of genital infection or injury, two had a history of prostatitis. Semen samples were collected throughout the year, 45 (23.0%) in spring (March to May), 48 (24.5%) in summer (June to August), 67 (34.2%) in autumn (September to November), and 36 (18.4%) in winter (December to February).

The median duration of abstinence was 3 days and the median time of liquefaction was 44 minutes. There were four subjects with azoospermia and therefore the association between serum micronutrient level and sperm motility and normal morphology could not be studied in these samples. Among these four cases of azoospermia, all had the diagnosis confirmed by repeat semen analysis and were referred to the Male Subfertility Clinic in Queen Mary Hospital for assessment and further investigations. Three individuals attended the clinic for assessment and one did not; two had non-obstructive azoospermia with the presence of varicocele and microdissection testicular sperm extraction with or without intracytoplasmic sperm insemination was discussed. One was diagnosed to be likely obstructive azoospermia and was scheduled for scrotal exploration. The median (range) semen volume was 2.5 (2.0-3.8) mL, the median sperm concentration was 30 (14-48) M/mL, the median PR motility was 27.5% (18%-34%), the median total motility (PR+NP) was 37% (30%-45%), and the median normal form was 2% (1%-4%). Both median semen volume and sperm concentration of the participants were within the normal range, but median motility and normal form were below the normal range.

Serum Folate and Vitamin B_{12}

Two samples were cancelled due to gross haemolysis. The median serum folate and vitamin B_{12} level was 7.60 (5.68-9.73) ng/mL and 448.50 (365-562.75) pg/mL, respectively, both within the normal range recommended by our laboratory. The majority of Chinese participants (97.4%) had a normal serum level of folate. There was no statistically significant association between serum folate (Table 2) and vitamin B_{12} (Table 3) levels respectively with each semen parameter.

Serum Zinc

One haemolysed sample was excluded from analysis. The median (range) serum zinc level was 12 (11-13) µmol/l. There was no statistically significant association between serum zinc level and each semen parameter (Table 4).

Table 1. Demographics of participants (n=196)

Demographics	Data*
Age (years)	37 (34-42)
21-30	18 (9.2%)
31-40	117 (59.7%)
41-50	51 (26%)
51-60	9 (4.6%)
61-70	1 (0.5%)
Occupation†	
Managers and administrators	12 (6.1%)
Professionals	16 (8.2%)
Associate professionals	63 (32.1%)
Clerks	30 (15.3%)
Service workers and shop sales workers	38 (19.4%)
Plant and machine operators and assemblers	18 (9.2%)
Elementary occupations	8 (4.1%)
Self-employed	4 (2.0%)
Unemployed	3 (1.5%)
Retired	1 (0.5%)
Missing data	3 (1.5%)
Smoking status	
Non-smoker	122 (62.2%)
Smoker	57 (29.1%)
Ex-smoker	17 (8.7%)
Disease	
Hypertension	9 (4.6%)
Diabetes	4 (2.0%)
Hyperlipidaemia	3 (1.5%)
Obesity	2 (1.0%)
Gout	2 (1.0%)
Hypothyroidism on T4 supplement	2 (1.0%)
History of mumps	12 (6.1%)
Varicocele	2 (1.0%)
Varicocele with operation done	1 (0.5%)
History of prostatitis	2 (1.0%)

Table 1. (cont'd)

Demographics	Data*
Season of semen collection‡	
Spring	45 (23.0%)
Summer	48 (24.5%)
Autumn	67 (34.2%)
Winter	36 (18.4%)
Days of abstinence	3 (3-5)
0-1	1 (0.5%)
2-3	100 (51.0%)
4-7	92 (46.9%)
>7	3 (1.5%)
Time of liquefaction (mins)	44 (33.5-55.0)
Within 1 hour	177 (90.3%)
1-2 Hours	19 (9.7%)
Semen parameters§	
Volume (mL)	2.5 (2.0-3.8)
Concentration (M/mL)	30 (14-48)
Motility PR¶ (%)	27.5 (18-34)
Total motility (PR+NP)¶ [%]	37 (30-45)
Normal form¶ (%)	2 (1-4)

* Data are shown as median (interquartile) or No. (%) of subjects

† Classified according to the International Labour Organization[30]

‡ Classified according to the Hong Kong Observatory

§ Determination according to the World Health Organization (2010) laboratory manual for the examination and processing of human semen, 5th edition[29] were employed as reference values. Normal reference values were semen volume of 1.5 mL, sperm concentration of 15 M/mL, motility PR of 32%, total motility (PR+NP) of 40%, and normal morphology of 4%

¶ Four semen samples were azoospermia and were excluded from analysis

Serum Magnesium

Low serum magnesium level was not found in any sample. The median (range) serum magnesium level was 0.92 (0.88-0.97) mmol/l. There was no statistically significant association between serum magnesium and semen volume, sperm concentration or motility (Table 5). Nonetheless there was a significant association between serum magnesium and normal morphology of sperm (p=0.02). Those who had a high serum magnesium level were less likely to have an abnormal form of sperms (odds ratio [OR]=0.13, 95% confidence interval [CI], 0.03-0.61, p=0.01) but were more likely to be older (OR=1.12, 95% CI, 1.02-1.24, p=0.02) [Table 5 and 6].

Smoking Status

Current smoker status was significantly associated with low semen volume compared with non-smoker and ex-smoker (Table 7).

Discussion

Our study demonstrates a significant association between serum magnesium and normal sperm morphology (p=0.02): a higher serum magnesium level was less likely to be associated with abnormal sperm morphology. The

Table 2. Serum folate level and semen parameters in Chinese infertile men (n=194)*

	Serum folate level (ng/mL)			p Value
	Deficient (<3.10)	Sufficient (3.10-20.50)	High (>20.50)	
Current smoking status				0.28
No	1 (0.5%)	134 (69.1%)	2 (1.0%)	
Yes	2 (1.0%)	55 (28.4%)	0	
Hypertension				1
No	3 (1.5%)	180 (92.8%)	2 (1.0%)	
Yes	0	9 (4.6%)	0	
Diabetes				1
No	3 (1.5%)	186 (95.9%)	2 (1.0%)	
Yes	0	3 (1.5%)	0	
Hyperlipidaemia				1
No	3 (1.5%)	186 (95.9%)	2 (1.0%)	
Yes	0	3 (1.5%)	0	
Obesity				1
No	3 (1.5%)	187 (96.4%)	2 (1.0%)	
Yes	0	2 (1.0%)	0	
Gout				1
No	3 (1.5%)	187 (96.4%)	2 (1.0%)	
Yes	0	2 (1.0%)	0	
Hypothyroidism on T4 supplement				1
No	3 (1.5%)	187 (96.4%)	2 (1.0%)	
Yes	0	2 (1.0%)	0	
History of mumps				1
No	3 (1.5%)	177 (91.2%)	2 (1.0%)	
Yes	0	12 (6.2%)	0	
Varicocele				1
No	3 (1.5%)	187 (96.4%)	2 (1.0%)	
Yes	0	2 (1.0%)	0	
Varicocele with operation				1
No	3 (1.5%)	188 (96.9%)	2 (1.0%)	
Yes	0	1 (0.5%)	0	
History of prostatitis				1
No	3 (1.5%)	187 (96.4%)	2 (1.0%)	
Yes	0	2 (1.0%)	0	
Season of collection				0.61
Spring	2 (1.0%)	43 (22.2%)	0	
Summer	0	46 (23.7%)	1 (0.5%)	
Autumn	1 (0.5%)	65 (33.5%)	1 (0.5%)	
Winter	0	35 (18.0%)	0	
Duration of abstinence (days)				0.66
0-1	0	1 (0.5%)	0	
2-3	2 (1.0%)	95 (49.0%)	2 (1.0%)	
4-7	1 (0.5%)	90 (46.4%)	0	
>7	0	3 (1.5%)	0	

Abbreviations: NP = non-progressive motility; PR = progressive motility

* Two blood samples were grossly haemolysed and were excluded from analysis

† Four semen samples were azoospermia and were excluded from analysis

Table 2. (cont'd)

	Serum folate level (ng/mL)			p Value
	Deficient (<3.10)	Sufficient (3.10-20.50)	High (>20.50)	
Time of liquefaction				1
Within 1 hour	3 (1.5%)	170 (87.6%)	2 (1.0%)	
1-2 Hours	0	19 (9.8%)	0	
Semen parameters				
Volume (mL)				
Normal	3 (1.5%)	172 (88.7%)	2 (1.0%)	1
Abnormal	0	17 (8.8%)	0	
Concentration (M/mL)				
Normal	3 (1.5%)	141 (72.7%)	1 (0.5%)	0.37
Abnormal	0	48 (24.7%)	1 (0.5%)	
Motility PR (%)†				
Normal	0	67 (35.3%)	0	0.48
Abnormal	3 (1.6%)	118 (62.1%)	2 (1.1%)	
Total motility (PR+NP) [%]†				
Normal	0	85 (44.7%)	0	0.17
Abnormal	3 (1.6%)	100 (52.6%)	2 (1.1%)	
Normal form (%)†				
Normal	0	56 (29.5%)	0	0.59
Abnormal	3 (1.6%)	129 (67.9%)	2 (1.1%)	

median level of each micronutrient studied was within the normal range, indicating that deficiency of these nutrients is rare in our locality. Of note, serum folate deficiency is common in western countries. We also demonstrated that smoking was significantly associated with low semen volume.

In our study, we demonstrated that a higher level of serum magnesium was significantly associated with a higher percentage of normal sperm morphology. Daily magnesium intake in the human diet is often below the daily requirement that may be even higher for many patients who engage in high levels of physical activity and / or who experience high levels of stress[31,32]. Thus, a relative hypomagnesaemia may occur despite the fact that the serum magnesium level is within the clinically physiological reference range.

A prospective pilot study was conducted by Kiss et al[33] and Viski et al[34] to evaluate the effectiveness of oral magnesium therapy on human semen parameters. They reported that 1 mg oral magnesium-citrate, administered daily for 3 continuous months, increased sperm volume, sperm count, motile sperm ratio, and normal morphology ratio. However, Závaczki et al[35] have conducted a randomised, placebo-controlled clinical pilot study among 20 men who suffered from idiopathic infertility to examine the effect of magnesium orotate on male idiopathic infertility, and shown that treatment at a dose of 3000 mg/day for 90 consecutive days led to neither a significant improvement in sperm variables nor an increased pregnancy rate in female partners of treated males compared with the controls.

Nonetheless, it does suggest that magnesium supplementation is advisable for patients if their daily dietary magnesium intake is less than the required amount, or if magnesium loss is increased due to chronic illness, high physical activity, or stress[35]. Among our participants, occupation could be a source of stress in the case of administrators and professionals, or a high degree of physical activity required by jobs such as labouring work; nonetheless the problem of infertility was also an important stressor. Magnesium is abundant in nature, found in green vegetables, chlorophyll, cocoa derivatives, nuts, wheat, seafood, and meat. Although further research on magnesium supplementation is needed, an increased dietary intake is a considerable alternative.

Table 3. Serum vitamin B_{12} level and semen parameters in Chinese infertile men (n=194)*

	Vitamin B_{12} level (pg/mL)			p Value
	Deficient (<187)	Sufficient (187-883)	High (>883)	
Current smoking status				0.77
No	1 (0.5%)	130 (67.0%)	6 (3.1%)	
Yes	0	56 (28.9%)	1 (0.5%)	
Hypertension				0.08
No	0	178 (91.8%)	7 (3.6%)	
Yes	1 (0.5%)	8 (4.1%)	0	
Diabetes				1
No	1 (0.5%)	183 (94.3%)	7 (3.6%)	
Yes	0	3 (1.5%)	0	
Hyperlipidaemia				1
No	1 (0.5%)	183 (94.3%)	7 (3.6%)	
Yes	0	3 (1.5%)	0	
Obesity				1
No	1 (0.5%)	184 (94.8%)	7 (3.6%)	
Yes	0	2 (1.0%)	0	
Gout				1
No	1 (0.5%)	184 (94.8%)	7 (3.6%)	
Yes	0	2 (1.0%)	0	
Hypothyroidism on T4 supplement				1
No	1 (0.5%)	184 (94.8%)	7 (3.6%)	
Yes	0	2 (1.0%)	0	
History of mumps				0.41
No	1 (0.5%)	175 (90.2%)	6 (3.1%)	
Yes	0	11 (5.7%)	1 (0.5%)	
Varicocele				1
No	1 (0.5%)	184 (94.8%)	7 (3.6%)	
Yes	0	2 (1.0%)	0	
Varicocele with operation				1
No	1 (0.5%)	185 (95.4%)	7 (3.6%)	
Yes	0	1 (0.5%)	0	
History of prostatitis				0.08
No	1 (0.5%)	185 (95.4%)	6 (3.1%)	
Yes	0	1 (0.5%)	1 (0.5%)	
Season of collection				0.21
Spring	0	41 (21.1%)	4 (2.1%)	
Summer	0	45 (23.2%)	2 (1.0%)	
Autumn	1 (0.5%)	65 (33.5%)	1 (0.5%)	
Winter	0	35 (18.0%)	0	
Duration of abstinence (days)				0.15
0-1	0	1 (0.5%)	0	
2-3	0	96 (49.5%)	3 (1.5%)	
4-7	1 (0.5%)	87 (44.8%)	3 (1.5%)	
>7	0	2 (1.0%)	1 (0.5%)	

Abbreviations: NP = non-progressive motility; PR = progressive motility

* Two blood samples were grossly haemolysed and were excluded from analysis

† Four semen samples were azoospermia and were excluded from analysis

Table 3. (cont'd)

	Vitamin B_{12} level (pg/mL)			p Value
	Deficient (<187)	Sufficient (187-883)	High (>883)	
Time of liquefaction				1
Within 1 hour	1 (0.5%)	167 (86.1%)	7 (3.6%)	
1-2 Hours	0	19 (9.8%)	0	
Semen parameters				
Volume (mL)				
Normal	1 (0.5%)	169 (87.1%)	7 (3.6%)	1
Abnormal	0	17 (8.8%)	0	
Concentration (M/mL)				
Normal	1 (0.5%)	140 (72.2%)	4 (2.1%)	0.53
Abnormal	0	46 (23.7%)	3 (1.5%)	
Motility PR (%)†				
Normal	0	65 (34.2%)	2 (1.1%)	1
Abnormal	1 (0.5%)	117 (61.6%)	5 (2.6%)	
Total motility (PR+NP) [%]†				
Normal	1 (0.5%)	82 (43.2%)	2 (1.1%)	0.34
Abnormal	0	100 (52.6%)	5 (2.6%)	
Normal form (%)†				
Normal	0	56 (29.5%)	0	0.13
Abnormal	1 (0.5%)	126 (66.3%)	7 (3.7%)	

Table 4. Serum zinc level and semen parameters in Chinese infertile men (n=195)*

	Serum zinc level (μmol/L)			p Value
	Deficient (<10)	Sufficient (10-19)	High (>19)	
Current smoking status				0.33
No	8 (4.1%)	130 (66.7%)	0	
Yes	2 (1.0%)	54 (27.7%)	1 (0.5%)	
Hypertension				0.11
No	8 (4.1%)	177 (90.8%)	1 (0.5%)	
Yes	2 (1.0%)	7 (3.6%)	0	
Diabetes				0.21
No	9 (4.6%)	181 (92.8%)	1 (0.5%)	
Yes	1 (0.5%)	3 (1.5%)	0	
Hyperlipidaemia				1
No	10 (5.1%)	181 (92.8%)	1 (0.5%)	
Yes	0	3 (1.5%)	0	
Obesity				1
No	10 (5.1%)	182 (93.3%)	1 (0.5%)	
Yes	0	2 (1.0%)	0	

Abbreviations: NP = non-progressive motility; PR = progressive motility
* One blood sample was haemolysed and were excluded from analysis
† Four semen samples were azoospermia and were excluded from analysis

Table 4. (cont'd)

	Serum zinc level (µmol/L)			p Value
	Deficient (<10)	Sufficient (10-19)	High (>19)	
Gout				1
No	10 (5.1%)	182 (93.3%)	1 (0.5%)	
Yes	0	2 (1.0%)	0	
Hypothyroidism on T4 supplement				1
No	10 (5.1%)	182 (93.3%)	1 (0.5%)	
Yes	0	2 (1.0%)	0	
History of mumps				1
No	10 (5.1%)	172 (88.2%)	1 (0.5%)	
Yes	0	12 (6.2%)	0	
Varicocele				1
No	10 (5.1%)	182 (93.3%)	1 (0.5%)	
Yes	0	2 (1.0%)	0	
Varicocele with operation				1
No	10 (5.1%)	183 (93.8%)	1 (0.5%)	
Yes	0	1 (0.5%)	0	
History of prostatitis				1
No	10 (5.1%)	182 (93.3%)	1 (0.5%)	
Yes	0	2 (1.0%)	0	
Season of collection				0.41
Spring	4 (2.1%)	41 (21.1%)	0	
Summer	1 (0.5%)	46 (23.6%)	0	
Autumn	3 (1.5%)	64 (32.8%)	0	
Winter	2 (1.0%)	33 (16.9%)	1 (0.5%)	
Duration of abstinence (days)				0.63
0-1	0	1 (0.5%)	0	
2-3	4 (2.1%)	94 (48.2%)	1 (0.5%)	
4-7	6 (3.1%)	86 (44.1%)	0	
>7	0	3 (1.5%)	0	
Time of liquefaction				
Within 1 hour	9 (4.6%)	166 (85.1%)	1 (0.5%)	
1-2 Hours	1 (0.5%)	18 (9.2%)	0	
Semen parameters				
Volume (mL)				
Normal	10 (5.1%)	167 (85.6%)	1 (0.5%)	0.64
Abnormal	0	17 (8.7%)	0	
Concentration (M/mL)				
Normal	7 (3.6%)	138 (70.8%)	1 (0.5%)	0.79
Abnormal	3 (1.5%)	46 (23.6%)	0	
Motility PR (%)[†]				
Normal	1 (0.5%)	65 (34.0%)	1 (0.5%)	0.08
Abnormal	9 (4.7%)	115 (60.2%)	0	
Total motility (PR+NP) [%][†]				
Normal	2 (1.0%)	82 (42.9%)	1 (0.5%)	0.11
Abnormal	8 (4.2%)	98 (51.3%)	0	
Normal form (%)[†]				
Normal	3 (1.6%)	54 (28.3%)	0	1
Abnormal	7 (3.7%)	126 (66.0%)	1 (0.5%)	

Table 5. Serum magnesium level and semen parameters in Chinese infertile men (n=196)

	Serum magnesium level (mmol/L)		p Value
	Sufficient (0.70-1.05)	High (>1.05)	
Median (interquartile range) age (years)	36 (33-42)	41 (38-45.5)	0.05
Current smoking status			0.72
No	133 (67.9%)	6 (3.1%)	
Yes	54 (27.6%)	3 (1.5%)	
Hypertension			0.35
No	179 (91.3%)	8 (4.1%)	
Yes	8 (4.1%)	1 (0.5%)	
Diabetes			1
No	183 (93.4%)	9 (4.6%)	
Yes	4 (2.0%)	0	
Hyperlipidaemia			1
No	184 (93.9%)	9 (4.6%)	
Yes	3 (1.5%)	0	
Obesity			1
No	185 (94.4%)	9 (4.6%)	
Yes	2 (1.0%)	0	
Gout			1
No	185 (94.4%)	9 (4.6%)	
Yes	2 (1.0%)	0	
Hypothyroidism on T4 supplement			1
No	185 (94.4%)	9 (4.6%)	
Yes	2 (1.0%)	0	
History of mumps			0.10
No	177 (90.3%)	7 (3.6%)	
Yes	10 (5.1%)	2 (1.0%)	
Varicocele			1
No	185 (94.4%)	9 (4.6%)	
Yes	2 (1.0%)	0	
Varicocele with operation			1
No	186 (94.9%)	9 (4.6%)	
Yes	1 (0.5%)	0	
History of prostatitis			1
No	185 (94.4%)	9 (4.6%)	
Yes	2 (1.0%)	0	
Season of collection			0.18
Spring	43 (21.9%)	2 (1.0%)	
Summer	47 (24.0%)	1 (0.5%)	
Autumn	61 (31.1%)	6 (3.1%)	
Winter	36 (18.4%)	0	0.18
Duration of abstinence (days)			0.25
0-1	1 (0.5%)	0	
2-3	98 (50.0%)	2 (1.0%)	
4-7	85 (43.4%)	7 (3.6%)	
>7	3 (1.5%)	0	

Abbreviations: NP = non-progressive motility; PR = progressive motility

* Four semen samples were azoospermia and were excluded from analysis

Table 5. (cont'd)

	Serum magnesium level (mmol/L)		p Value
	Sufficient (0.70-1.05)	High (>1.05)	
Time of liquefaction			0.21
Within 1 hour	170 (86.7%)	7 (3.6%)	
1-2 Hours	17 (8.7%)	2 (1.0%)	
Semen parameters			
Volume (mL)			
Normal	170 (86.7%)	9 (4.6%)	1
Abnormal	17 (8.7%)	0	
Concentration (M/mL)			
Normal	139 (70.9%)	8 (4.1%)	0.46
Abnormal	48 (24.5%)	1 (0.5%)	
Motility PR (%)*			
Normal	65 (33.9%)	2 (1.0%)	0.50
Abnormal	118 (61.5%)	7 (3.6%)	
Total motility (PR+NP) [%]*			
Normal	81 (42.2%)	5 (2.6%)	0.52
Abnormal	102 (53.1%)	4 (2.1%)	
Normal form (%)*			
Normal	52 (27.1%)	6 (3.1%)	0.02
Abnormal	131 (68.2%)	3 (1.6%)	

Table 6. Correlation between having sufficient / high serum magnesium level with morphology of sperm and age

Variable	Odds ratio (95% confidence interval)	p Value
Morphology of sperm [Reference group: normal form (%)]	0.13 (0.03-0.61)	0.01
Age	1.12 (1.02-1.24)	0.02

Of note, our study shows that the majority of Chinese participants (97.4%) had a normal serum level of folate, contrary to western studies, and folate deficiency was rare; hence echoing the studies by Tso and Wong[36] and Lee et al[37,38]. These data revealed that there is a very low incidence of folate deficiency in Hong Kong Chinese: the Chinese style of food consumption in Hong Kong is characterised by a high daily intake of leafy green vegetables, in addition to soybean, green tea, and to a lesser extent animal liver, all of which represent rich sources of folate[39-42].

Among the participants, the median normal morphology of sperm was below the normal range. It is to be determined whether an increased dietary intake or supplementation of magnesium can improve these semen variables and this will be the subject of our future research.

In our study, smoking was significantly associated with low semen volume (p=0.01). The mechanism whereby cigarette smoking affects sperm function is not well understood. Some studies have shown that smoking has a detrimental effect on sperm quality, most significantly sperm concentration, motility, and morphology[43-47]. Since cigarette smoke contains many substances including nicotine, carbon monoxide, heavy metals, benzopyrene, dimethylbenzanthracene dimethylnitrosamine, naphthalene and metanaphtalene[48], smoking can increase inflammatory agents and effect sperm genome and gonads and failure in sperm-ovum fecundation and thus decrease fertility[49]. Contrary to this though, some studies have shown no

Table 7. Current smoking status and semen parameters in Chinese infertile men (n=196)

Semen parameters	Current smoking status		p Value
	Non-smokers and ex-smokers	Smokers	
Volume (mL)			0.01
Normal	132 (67.3%)	47 (24.0%)	
Abnormal	7 (3.6%)	10 (5.1%)	
Concentration (M/mL)			0.32
Normal	107 (54.6%)	40 (20.4%)	
Abnormal	32 (16.3%)	17 (8.7%)	
Motility PR (%)*			0.63
Normal	46 (24.0%)	21 (10.9%)	
Abnormal	90 (46.9%)	35 (18.2%)	
Total motility (PR+NP) (%)*			0.54
Normal	59 (30.7%)	27 (14.1%)	
Abnormal	77 (40.1%)	29 (15.1%)	
Normal form (%)*			0.18
Normal	45 (23.4%)	13 (6.8%)	
Abnormal	91 (47.4%)	43 (22.4%)	

Abbreviations: NP = non-progressive motility; PR = progressive motility
* Four semen samples exhibited azoospermia and were excluded from analysis

association between smoking and sperm quality[50,51], or sperm function[52].

The impact of cigarette smoking on male fertility thus remains a highly controversial issue. Since male smokers are very susceptible to oxidative damage induced by free radicals, infertile men who smoke cigarettes should be advised to quit given the potential adverse effects of seminal oxidative stress[48].

Strengths and Limitations

Our study is the first research study in a Chinese population in our locality to examine the relationship of serum folic acid, vitamin B_{12}, zinc, and magnesium, to semen parameters in infertile couples. We have demonstrated a significant association between serum magnesium and sperm normal morphology. Since a higher serum magnesium level is associated with a higher percentage of normal sperm morphology, this will aid in further exploration of potential treatments for male subfertility.

There were some limitations of our study. Semen parameters vary between samples from the same individual, and in our study only one semen sample was collected and studied from each participant. Hence it might not truly reflect the quality of semen of the study subject. In addition the serum micronutrient level will vary over time due to dietary intake and physical consumption. We therefore collected the semen and blood samples on the same day assuming that the serum micronutrients studied were involved in the spermatogenesis of semen samples being analysed.

A multi-centre WHO study on the influence of varicocele on fertility parameters demonstrated that varicocele is associated with impaired testicular function and infertility[53]. Whether surgery can help improve semen quality remains controversial. One of the participants in our study had a history of varicocele surgery and the remaining two were identified to have varicocele upon examination in the male subfertility clinic; since there was no routine physical examination of men in our subfertility clinic, the prevalence of varicocele might have been underestimated.

Conclusion

Our study is the first research study in a Chinese population in our locality to examine the relationship of serum folic acid, vitamin B_{12}, zinc, and magnesium to semen parameters in infertile couples. We demonstrated a significant association between serum magnesium and normal morphology of sperm as well as the detrimental effect of smoking on semen volume, contributing to male infertility. Male factor subfertility is a multifactorial disorder. Nutritional factors, unlike genetic factors, can be adjusted by altering dietary intake. Whether an improvement in normal sperm morphology following magnesium supplementation or increased dietary intake will result in an increase in pregnancy rates remains to be established. This should further stimulate research on nutrition and environmental factors in the pathogenesis and prevention of fertility disorders.

Declaration

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