

Regional Differences in Semen Quality Between Guangzhou and Hong Kong in South China: A Cross-Sectional Study

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Research

Keywords: Semen quality, geography, sperm concentration, sperm motility, sperm morphology

Posted Date: June 30th, 2020

DOI: <https://doi.org/10.21203/rs.3.rs-37222/v1>

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Abstract

Background: The geographic variation in semen quality has been reported in many countries. However, the situation in China is still unknown. To answer this question, we examined the variation in semen parameters between two cities in south China: Guangzhou (GZ) and Hong Kong (HK).

Methods: Semen analysis data of 13507 subjects from GZ and 1106 subjects from HK were retrospectively analysed. In order to control for referral bias, we selected two well-defined subpopulations, namely, subpopulation Ⅰ: male partners of women with an underlying cause of subfertility, which is assumed to be similar to the general male population; subpopulation Ⅱ: normozoospermic men, which is assumed to represent men with normal spermatogenesis, sperm maturation and sperm transportation. In addition, we have also chosen to control for two well-recognized confounding variables, namely, age and abstinence time by using a case matching design and generalised additive models (GAM).

Results: In using the case matching design, subjects from different cities with similar age and abstinence time were matched. The results of the paired T test showed that sperm concentration and motility of subjects in HK were significantly lower than that of subjects in GZ in both subpopulation Ⅰ and Ⅱ ($P < 0.05$); on the other hand, the sperm morphology of subjects in HK was significantly lower than that of subjects in GZ in subpopulation Ⅰ ($P < 0.05$) but not in subpopulation Ⅱ ($P > 0.05$). The results of GAM model showed that the sperm parameters were varied by age and/or abstinence time, however, the sperm concentration, motility and morphology of subjects in HK were significantly lower than that of subjects in GZ in both subpopulation Ⅰ and Ⅱ ($P < 0.05$).

Conclusions: The semen quality of subjects in HK was significantly worse than that of subjects in GZ, which may be caused by environmental and lifestyle factors, given that the study subjects in both cities of the present study are of the same ethnic origin.

Trial registration: Not applicable.

Background

The controversy over trends in semen quality has persisted over the past decades, with some studies reporting a downward trend [1–4], while others did not [5–7]. Although the secular trends in semen quality was still inconclusive, the regional differences of semen quality were apparent according to those previous reports [8].

In order to demonstrate regional variation in semen quality between different places, Swan et al. first used the data published in previous literatures and re-analyzed the data and found that the sperm concentration of men in European countries was higher than that of men in non-Western countries [9]. Then, Jorgensen et al. [10] conducted a coordinated and quality controlled cross-sectional study including 1082 fertile men from four European countries. The results showed that there were significant differences in sperm concentration and motility among Danish, Finnish, Scottish and French men [10]. After that, Swan et al. published a similar study in USA, which investigated fertile men and obtained similar results [11]. Later, Iwamoto et al. compared semen analysis data of Japanese men and previous data reported in the European study [10]. The result

showed that sperm concentration and motility of Japanese men was lower than that of men from European countries [12]. Regarding the geographic variation in semen quality, the genetic background, lifestyle and environmental factors were all considered as possible underlying reasons [13–17]. A number of researchers suggested that the lifestyle factors and environmental factors, rather than an accumulation of genomic structural defects, are the main causes of geographic variation in semen quality [18]. However, till now, the situation in China is still unclear.

In order to examine the geographic variation of semen quality in China and its possible contributory, the semen analysis data of men in Guangzhou (GZ) and Hong Kong (HK) were collected and compared. GZ and HK are two coastal cities located in Pearl River Delta in south China and the vast majority of the populations of these two cities are Cantonese, however, HK used to be colonized by United Kingdom and the lifestyles of the residents in these two cities are rather different. Most of the GZ residents adopted a typical Chinese lifestyle, whereas most of the HK resident have a mixed westernised and Chinese lifestyle. Studies have shown that the lifestyles of HK resident are “less-healthy” including reduced physical activities, more fatty diet, staying up late and generally more stressed [19, 20]. Moreover, the living environment in HK are also different from GZ. The population density and the heavy metal pollution in HK are relatively higher than GZ[21, 22]. The particulate matter (PM) in the air in HK is lower than in GZ [23–25]. Therefore, GZ and HK are two cities which are particularly suited to investigate the effect of lifestyle and environmental factors on semen quality.

In the present study, the potential referral bias was controlled by investigating two subpopulations: subpopulation Ⅰ: male partners of women with an underlying cause of subfertility; subpopulation Ⅱ: normozoospermic men. Furthermore, the male age and abstinence time were controlled by case-matching design and generalised additive models (GAM) model in the analysis. The results generated from this study can provide insight into the impact of lifestyle and environmental factors on semen quality.

Material And Methods

Source population

Semen analysis (SA) data from subjects who underwent routine fertility assessment between 1 January 2012 and 31 December 2016 were retrospectively collected from the following two reproductive centres: 1. Centre for Reproductive Medicine of NanFang Hospital, Southern Medical University (GZ); 2. Assisted Reproductive Technology Unit of the Prince of Wales Hospital, the Chinese University of Hong Kong (HK). Besides the SA data, additional relevant information such as male age, abstinence time and infertility cause were also collected, if available. For individuals who had more than one SA report available, the data from the first report was included in the study.

Subpopulations

In order to control for the potential referral bias, two subpopulations were investigated from the entire source population:

Subpopulation Ⅱ: male partners of women who had a proven cause of infertility, which would have characteristics close to the general male population according to Fisch et al. [26]. The female cause infertility including 1. Bilateral Fallopian tubes absent or blocked; 2. Endometriosis; 3. Anovulation; 4. Pelvic adhesions; 5. Female congenital reproductive tract malformation; 6. Asherman's syndrome.

Subpopulation Ⅲ: Normozoospermic men, which represented men with normal function of spermatogenesis, sperm maturation and sperm transportation. Normozoospermia was defined according to the World Health Organization (WHO) reference values: concentration $\geq 15 \times 10^6/\text{mL}$, motility $\geq 40\%$, and morphology $\geq 4\%$ [27].

Semen Analysis

Both participating centers in our study performed SA according to WHO guidelines (version V) [27]. Semen samples were collected by masturbation directly into a polyethene jar and analysed within one-hour post ejaculation after liquefaction. In the assisted conception unit of Prince of Wales Hospital (HK), the assessment of sperm concentration was performed by using hemocytometers with improved Neubauer ruling and a phase contrast microscope (magnification of $\times 200$). The sperm motility was assessed by using wet preparations made with a 10 μl drop of semen and a 22 \times 22 mm coverslip. In the reproductive center of NanFang Hospital (GZ), a computer-aided semen analysis (CASA) system (Sperm Class Analyzer (SCA) version 4.0.0.5, Microptic S.L., Barcelona, Spain) and a four-chamber slide (Leja, Amsterdam, Netherlands) were used to perform the assessment of sperm concentration and motility. For sperm morphology, sperm slides stained by Diff-Quik staining kit (Dade Behring AG, Dürdingen, Switzerland) were evaluated according to Tygerberg Strict Criteria under a microscope with an oil immersion $\times 100$ objective (OLYMPUS BX43, Tokyo, Japan) in both centers. When assessing the sperm concentration, motility and morphology, at least 200 sperm were examined on each occasion and duplicate assessments were conducted in both centers.

Inter-laboratory Variation In Semen Analysis

Both participating centers performed monthly internal quality control for semen analysis. For external quality control, the assisted conception unit of Prince of Wales Hospital (HK) joined in the United Kingdom National External Quality Assessment Service (UK NEQAS), whereas the reproductive center of NanFang Hospital (GZ) joined in the external quality control scheme in China. To assess the inter-laboratory variation between the two participated centers, sixteen fixed semen samples were distributed to both centers to evaluate the variation of sperm concentration and morphology assessment, and sixteen motility videos were similarly distributed to measure the variation of sperm motility assessment.

Statistical analysis

R software (R Development Core Team, 2004) was used in data analysis, and P-values of < 0.05 were considered to be significant. Descriptive statistics were performed to analyze the mean and standard deviation (SD) of clinical characteristics and semen parameters of participants. The inter-laboratory

variations of semen analysis between the two centers were calculated by the two-way random models' intra-class coefficient (ICC (2,1)). Student t-test was used in the comparison of clinical features and semen parameters of subjects between GZ and HK. To further control for the potential bias caused by male age and abstinence time, a 1:1 case matching design was used. The subjects from both centers with similar age (± 1 year) and abstinence time (± 1 day) were randomly selected, and paired t-test was performed to compare the sperm parameters between the two cities.

In order to virtualize the variation of semen parameters with the increased age and abstinence time in different cities, GAM models were used [28]. The smoothed function was performed on variable "male age" and "abstinence time". Box-Cox Transforms[29] were applied to skewed variable including sperm concentration, motility and morphology in subpopulations \square and \square . Package "mgcv" in R was used for GAM computation, and package "visreg" was used for model visualisation.

Results

Clinical characteristics and semen parameters of the study populations

There are 13507 and 1106 SA data collected from the two reproductive centres in GZ and HK respectively. Among them, there were 2355 subjects from GZ and 374 subjects from HK with proven female infertility, and constituted subpopulation \square ; There were 3142 subjects from GZ and 313 subjects from HK with normal semen parameters and constituted subpopulation \square . The comparison of clinical characteristics and semen parameters of the source population, subpopulations \square and \square are shown in Table 1. The subjects in HK were significantly older, with shorter abstinence time, lower sperm concentration and motility when compared with the subjects in GZ in the source population and both subpopulations \square and \square . However, the sperm morphology of subjects in HK were significantly lower than that of subjects in GZ in the source population and subpopulation \square , but not subpopulation \square .

Table 1

A comparison of clinical characteristics and semen parameters of different populations between subjects from Hong Kong (HK) and Guangzhou (GZ).

Clinical characteristics/ semen parameters	Source population			Subpopulation Ⅰ			Subpopulation Ⅱ		
	HK	GZ	P Value	HK	GZ	P Value	HK	GZ	P Value
Sample size	1106	13507		374	2355		313	3142	
Male age (years)	38.6 ± 6.5	33.6 ± 5.9	Ⅰ0.001	38.9 ± 5.4	34.0 ± 5.4	Ⅰ0.001	38.1 ± 5.44	33.7 ± 5.7	Ⅰ0.001
Abstinence time (days)	4.6 ± 3.9	4.9 ± 2.6	0.002	4.2 ± 1.4	4.6 ± 1.4	Ⅰ0.001	4.1 ± 1.4	4.4 ± 1.5	0.002
Concentration (× 10 ⁶ / ml)	25.5 ± 27.2	73.8 ± 40.1	Ⅰ0.001	26.1 ± 27.7	81.3 ± 48.6	Ⅰ0.001	43.8 ± 25.1	86.9 ± 51.6	Ⅰ0.001
% Motile sperm	42.6 ± 15.7	49.2 ± 20.3	Ⅰ0.001	30.4 ± 21.8	56.1 ± 20.3	Ⅰ0.001	51.9 ± 9.5	64.5 ± 13.9	Ⅰ0.001
% Normal morphology	3.4 ± 3.1	3.4 ± 2.2	0.003	3.5 ± 3.1	4.3 ± 2.1	Ⅰ0.001	6.2 ± 2.6	6.1 ± 1.6	0.37
Results shown are mean ± SD									

Comparison of semen parameters between case-matching subjects from HK and GZ in subpopulation Ⅰ and Ⅱ

There were 370–370 (HK-GZ) and 311–311 (HK-GZ) subjects who were matched by age and abstinence time, selected from subpopulation Ⅰ and Ⅱ, respectively. The comparison of clinical characteristics and semen parameters between the case-matching subjects from the two cities are shown in Table 2. There was no significant difference in age and abstinence time between the case-matching subjects from the two cities. The sperm concentration and motility of subjects in HK were significantly lower than that of case-matching subjects in GZ in both subpopulation Ⅰ and Ⅱ ($P < 0.05$). The sperm morphology of subjects in HK was significantly lower than that of case-matching subjects in GZ in subpopulation Ⅰ ($P < 0.05$), but not Ⅱ ($P > 0.05$).

Table 2

A comparison of clinical characteristics and semen parameters of the matched-control cases between subjects from Hong Kong (HK) and Guangzhou (GZ)

Study population	Clinical characteristics/ semen parameters	HK	GZ	P Value
Subpopulation Ⅱ	Sample size	370	370	-
	Male age (years)	38.8 ± 5.2	38.8 ± 5.1	0.59
	Abstinence time (days)	4.2 ± 1.4	4.2 ± 1.4	0.11
	Concentration ($\times 10^6$ / ml)	26.1 ± 27.7	78.4 ± 45.4	Ⅱ0.001
	% Motile sperm	30.7 ± 21.7	55.5 ± 20.7	Ⅱ0.001
	% Normal morphology	3.5 ± 3.1	4.4 ± 2.2	Ⅱ0.001
Subpopulation Ⅲ	Sample size	311	311	-
	Male age (years)	38.0 ± 5.1	38.0 ± 5.1	0.16
	Abstinence time (days)	4.1 ± 1.4	4.1 ± 1.4	0.51
	Concentration ($\times 10^6$ / ml)	43.9 ± 25.1	88.0 ± 47.4	Ⅱ0.001
	% Motile sperm	52.0 ± 9.5	63.5 ± 14.1	Ⅱ0.001
	% Normal morphology	6.3 ± 2.6	6.0 ± 1.3	0.06
Results shown are mean ± SD				

GAM analysis for the variation of semen parameters in two subpopulations from HK and GZ

The association between the semen parameters, age, abstinence time and cities were first analysed by univariate GAM models [30]. To adjust for the interaction between variables, the significant associations identified by the univariate GAM model were subsequently analysed in multivariate GAM. The results of univariate and multivariate GAM are shown in Supplementary Table 1. The patterns of the association in multivariate GAM are shown in Fig. 1 and described as followed: (1) In subpopulation Ⅱ, although the sperm concentration was significantly associated with age and abstinence time, the overall sperm concentration of subjects in GZ was significantly higher than that of subjects in HK ($P < 0.001$); the sperm motility and morphology were significantly associated with age, but the overall sperm motility and morphology of subjects in GZ were significantly higher than that of subjects in HK ($P < 0.001$). (2) In subpopulation Ⅲ, although the sperm concentration was significantly associated with abstinence time, the overall sperm concentration of subjects in GZ was significantly higher than that of subjects in HK ($P < 0.001$); the sperm motility was significantly associated with age, but the overall sperm motility of subjects in GZ was significantly higher than that of subjects in HK ($P < 0.001$); the sperm morphology in subpopulation Ⅲ was not significantly associated with age or abstinence time, however, it was only significantly associated with the cities ($P < 0.001$).

Inter-laboratory Variation Of Semen Analysis

The ICC of the assessment of sperm concentration, motility and morphology between the two participated centers were 0.85, 0.85 and - 0.24 respectively.

Discussion

In the present study, we examined the geographic variation of semen quality in two different cities in southern China – GZ and HK. The results show that the sperm concentration, motility and morphology of subjects in HK was worse than that of subjects in GZ. To the best of our knowledge, this is the first study reporting the geographic variation in semen quality in China.

When analyzed the raw data, we found that the sperm concentration and motility of subjects in HK were significantly lower than that of subjects in GZ, in the source population and subpopulation I and II . After controlled for the male age and abstinence time by case matching design in subpopulations I and II , the results were still the same. When further analysis was performed with the use of GAM models, the results showed that the sperm concentration and motility of subjects in GZ were significantly higher than of subjects in HK, although the sperm parameters were affected by age and/or abstinence time. Our results indicated that just like the situation in Europe [10], USA [11] and Japan [12], significant geographic variation in sperm concentration and motility also existed in China.

When analyzing the raw data regarding sperm morphology, the results showed that subjects in HK have significantly lower normal sperm morphology than that of subjects in GZ in the source population and subpopulation I , but not in subpopulation II . This result regarding the subpopulation II has been further confirmed by both analyses of case matching design and GAM model. However, in subpopulation II , the result of GAM model and case matching design was inconsistent. The analyses of GAM model showed subjects in GZ had significantly higher normal sperm morphology than that of subjects in HK, whereas the results of cases matching design did not show any significant difference. It should be noted that the analysis based on GAM model involved a larger sample size than the case matching design and the GAM model examined the non-linear association between variables. Our results suggested that the sperm morphology may be higher in the general male population in GZ when compared with that of general male population in HK. There is no significant difference in mean sperm morphology between men with normal spermiogram in HK and GZ. However, the sperm morphology of men with normal spermiogram in these two cities may have non-linear associations. Regarding the regional differences in sperm morphology, previous studies also reported conflicting results. Iwamoto et al. showed that the sperm morphology of men in Japan was significantly lower than that of European men [12]. However, Jorgensen et al. [10] and Swan et al. [11] did not find any significant differences in sperm morphology in different cities of European countries and USA.

When comparing the semen parameters of general population (subpopulation I) in south China with the semen parameters of general population from elsewhere, it appears that the mean (\pm SD) sperm concentration of general population in GZ ($81.3 \pm 48.6 \times 10^6/\text{ml}$) were relatively higher than that of subjects in Sichuan area (south-west China) ($65.6 \pm 46.9 \times 10^6/\text{ml}$) [31] and Denmark ($60 \pm 55 \times 10^6/\text{ml}$)[32], however, the mean (\pm SD) sperm concentration of subjects in HK was relatively lower ($26.1 \pm 27.7 \times 10^6 /\text{ml}$) than that of

subjects in Sichuan area and Denmark [31, 32]. The mean (\pm SD) sperm motility of general population in GZ ($56.1 \pm 20.3\%$) was comparable with that of general male population in Sichuan ($53.8 \pm 20.5\%$), but lower than that of subjects in Denmark ($65 \pm 15\%$), whereas the mean (\pm SD) sperm motility of general population in HK ($30.4 \pm 21.8\%$) was relatively lower than general male population in Sichuan and Denmark [31, 32]. Regarding the sperm morphology, the mean (\pm SD) normal morphology of general population in GZ ($4.3 \pm 2.1\%$) and HK ($3.5 \pm 3.1\%$) are both lower than that of men in Sichuan ($9.5 \pm 8\%$) [31].

Taken together, the results of our study suggest that the sperm quality of subjects in HK was worse than that of subjects in GZ. It has been recognized that regional differences in semen quality may be due to the difference in genetic background, as well as environment and lifestyle factors [8]. In this study, we investigated Cantonese men from two closely located cities in south China. The ethnic origin of the investigated subjects is the same. However, they have apparently different living environment and lifestyles [19–25]. It seems likely that the differences observed are caused by lifestyle and environmental factors.

There were several strengths in this study. Firstly, the regional differences in semen quality in south China has been confirmed by both subpopulation \boxtimes and \boxtimes , which represented the general male population and men with normal spermatogenesis and sperm maturation, respectively. In the previous relevant publications, only men with proven fertility were examined [10–12]. Secondly, we have used both case-matching design and GAM model to control for the potential bias caused by age and abstinence time, which had not been controlled in previous studies.

There were several limitations in our study: Firstly, the assessment of inter-laboratory variation was not conducted during the period of SA assessment due to the retrospective nature of this study. Secondly, the ICC value regarding morphology between the two laboratories were relatively low (-0.24). According to Landis [33], the coefficient values of 0.01 indicated “poor” agreement, 0.01 to 0.20 indicated “slight” agreement, 0.21 to 0.40 indicated “fair” agreement, 0.41 to 0.60 indicated “moderate” agreement, 0.61 to 0.80 indicated “substantial” agreement and 0.81 to 1.00 indicated “almost perfect” agreement. Therefore, the result regarding morphology should be viewed with caution.

Conclusion

Our study found significant differences in semen parameters between HK and GZ, two cities in south China. The differences were still apparent after controlling for four confounding variables, including laboratory standards, referral bias, age and abstinence time. The semen quality of male subjects in HK appears to be lower than that of male subjects in GZ. It seems likely that environmental and lifestyle factors contributed to the differences in sperm parameters between the male subjects from two different cities but with the same ethnic origin.

Abbreviations

CASA
Computer-Aided Semen Analysis; GAM:Generalised Additive Models; ICC:Intra-Class Coefficient; SA:Semen Analysis; SCA:Sperm Class Analyzer; SD:Standard Deviation; UK NEQAS:United Kingdom National External

Quality Assessment Service; WHO:World Health Organization.

Declarations

Acknowledgements

The authors would like to thank Tarah Waters of the Chinese University of Hong Kong for her help on language editing.

Funding

The authors have no funding sources to report.

Availability of data and materials

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

SX performed the data complication, data analysis and wrote the manuscript. CPSC collected the SA data in Prince of Wales Hospital. DYLC reviewed and revised the manuscript. QS collected SA data in NanFang Hospital. TCL designed the study, contributed to writing of the manuscript and revising the final draft. All authors have approved of the final article.

Ethics approval and consent to participate

This study was approved by the Joint Chinese University of Hong Kong – New Territories East Cluster Clinical Research Ethics Committee (REC No.: 2015.491) and Medical Ethics committee of NanFang Hospital of Southern Medical University (No.: NFEC-2019-026). No consent was necessary in the present study.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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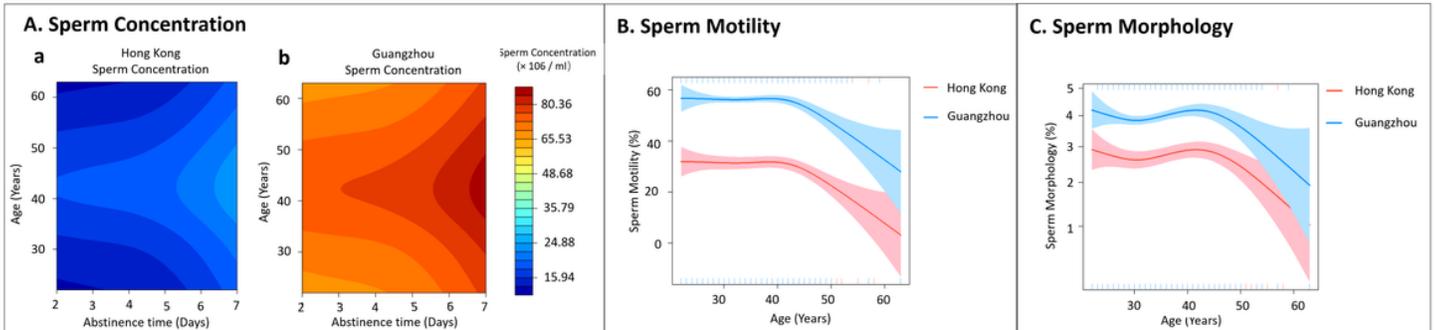
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Figures

Subpopulation I



Subpopulation II

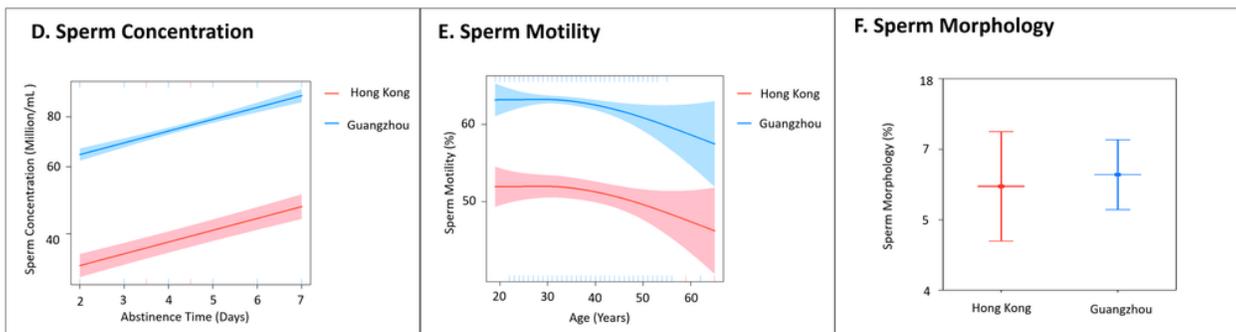


Figure 1

Variation in semen parameters with increased intensity of age and abstinence time of men from different cities in subpopulation I and II. The associations were evaluated by the GAM model, and only the significantly associated parameters are shown in the figures. Figure (1A), The variation of sperm concentration with increased age and abstinence time in Hong Kong (a) and Guangzhou (b) in subpopulation I. The colour bar represents the intensity of sperm concentration, with the cold colour representing lower sperm concentration and warm colour representing higher sperm concentration. Figure (1B) and Figure (1C), The variation of sperm motility and morphology with increased male age in subjects of Hong Kong (red) and Guangzhou (blue) in subpopulation I respectively. The solid lines show the regression lines of sperm motility and morphology based on the GAM model, respectively. The shadows represent the 95% confidence interval. Figure (1D) and Figure (1E), The variation in sperm concentration and motility with increased abstinence time of subjects in Hong Kong (red) and Guangzhou (blue) in subpopulation I. The solid lines show the regression lines of sperm concentration and motility based on the GAM model, respectively. The shadows represent the 95% confidence interval. Figure (1F) show the difference in sperm morphology of subjects in Hong Kong (red) and Guangzhou (blue) in subpopulation I. The middle, upper and lower horizontal lines represent the median, 25% and 75% quantile, respectively.

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