

# Sperm Quality and Environment: A Retrospective, Cohort Study among 21,715 Semen Samples in a Southern Province of China

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## Abstract

To explore the differences of male semen parameters in different seasons of the year, so as to explore the potential climatic factors affecting spermatogenesis and male reproductive ability, we retrospectively analyzed 21,715 semen analysis data from January 2018 to February 2021, grouped by year and season, and finally the relationships among semen parameters and semen and meteorological parameters were compared. Environmental exposures prior to 3 months were analyzed and correlation analysis was performed. The semen concentration decreased year by year ( $p < 0.01$ ). However, the Progressive motility (PR) and total PR number had been increased ( $p < 0.01$ ). There were statistical differences in sperm parameters which include semen volume, sperm concentration, total sperm number, progressive motility (PR), total PR number and total motility in different seasons, winter and spring were better than summer and autumn ( $p < 0.01$ ). Total sperm number and sperm concentration were positively correlated with PR ( $R = 0.420$ ,  $R = 0.440$ ,  $p < 0.01$ ). There was no correlation between daylight duration and semen parameters. Sperm parameters were positively or negatively correlated with environmental temperature, air pressure or humidity which had an overall effect on semen quality. It is suggested that seasonal factors should be considered when evaluating male reproductive ability. Besides referring to conventional semen parameters, other factors such as season and climate should also be considered.

## Keywords

Infertility, Seasonal Sperm Pattern, Semen Quality, Climatic Factors, Correlation Analysis

## 1. Introduction

In recent years, many studies have shown that the quality of male semen has a downward trend year by year, leading to an increasing proportion of male factors with reproductive disorders [1] [2] [3]. The internal factors causing the decline of male semen quality mainly include genetic factors, anatomical and physiological factors, and the external factors include environmental factors, lifestyle factors, mental factors and so on [4] [5]. Studies have shown that climate factors and air pollution may affect male reproductive ability. Many studies are committed to the impact of exposure to environmental chemicals and poisons on male reproductive health, like smoking, occupational Lead (Pb), traffic pollution such as nitrogen oxides, sulfur compounds, and sulfur oxides [6] [7] [8] [9]. In the past 100 years, the global average temperature has risen by 0.74°C, and the average temperature in China has risen by 0.79°C. In the past 30 years, industry in China has developed rapidly, which results in increasing environmental pollution, especially the air and water pollution caused by energy utilization and industrial production [10].

Various researchers demonstrate that changes in temperature and photoperiod are partly responsible for the periodic changes in sperm parameters [11]. Significant geographic differences in sperm concentration have been reported in Europe, North America, Asia, and Africa [4] [12]. Human and animal studies have shown that male semen quality including quantity, vitality, and fertility has a statistical difference from season to season [13] [14] [15] [16].

Clinically, the semen traits and parameters of the same patient are apparently different in various testing periods, including the total sperm count, sperm concentration, total motility, progressive motility (PR) and so on. This study aims to explore the differences of male semen parameters in different seasons of a year, so as to explore the potential influencing factors of spermatogenesis and male reproductive ability.

## 2. Materials and Methods

### 2.1. Patient Population

A retrospective analysis of 22,756 semen collected from Third Affiliated Hospital of Sun Yat-Sen University from January 1st, 2018 to February 28th, 2021. These 22,756 male patients were all patients who came to our hospital for semen examination in the past three years, including physical examination, premarital examination, infertility, etc. Patients were excluded if they have reproductive and developmental abnormalities, infectious factors, and systemic diseases which affected sperm quality such as diabetes, genitourinary dysplasia. Azoospermia, haemospermia, oligozoospermia, asthenozoospermia, oligosasthenozoospermia (total sperm number less than  $5 \times 10^6$  per ml) was also excluded according to the “WHO Laboratory Manual for the Examination and Processing of Human Semen (5th edition)” which established by World Health Organization (WHO) published in 2010 (WHO 2010 guidelines) [17]. Finally, 21,715 results

were selected.

## 2.2. Semen Collection and Analysis

According to the WHO 2010 guidelines, all patients abstained from sex for 2 - 7 days and masturbated to extract sperm. All semen samples were discharged in a sterile container and sent to the laboratory for testing within 30 minutes. After liquefaction, semen analysis was performed within 2 hours by the same laboratory technician using the Makler Counting Chamber (Saifi Medical Devices Co., Ltd. Haifa, Israel). Sperm parameters including ejaculate volume, PH, sperm concentration, total sperm number, total motility percent, and PR were assessed according to WHO 2010 guidelines.

## 2.3. Climate Data

The meteorological data of Guangzhou in recent three years (from Sep. 2017 to Feb. 2021) including temperature (°C), humidity (%), atmospheric pressure (hPa), and sunlight duration (hour/d) were obtained from the Meteorological Data Network of China (<http://data.cma.cn>).

Guangdong Province is a subtropical monsoon climate zone [18] [19], spring is warm and rainy, summer is hot and humid, winter is mild and dry, the four seasons are relatively distinct. According to the characteristics of subtropical climate, four seasons in a year are divided as follow: December, January and February are winter; March, April and May are spring; June, July and August are summer; and September, October and November are autumn.

## 2.4. Statistical Analysis

Statistical analyses were performed by using Statistical Package for Social Sciences (SPSS for Windows, version 25.0, SPSS Inc., Chicago, IL, USA). Scatter diagram production and its statistical analysis were performed by R software version 3.6.2. The clinical data on general characteristics are expressed as the mean  $\pm$  standard deviation. Comparisons of all semen parameters were performed using Mann-Whitney U test for comparison of two independent groups as they showed non-normal distributions. Correlations were calculated between the variables of interest. Spearman correlation test was used for correlation analysis. P value  $< 0.05$  was considered as statistically significant difference.

# 3. Results

## 3.1. Semen Data

We analyzed a total of 21,715 routine semen reports (**Table 1**) from January 2018 to February 2021, with a mean age of  $31.19 \pm 5.58$ , a sperm concentration of  $42.34 \pm 28.50 \times 10^6/\text{ml}$ , total motility of  $0.51 \pm 0.22$  and progressive motility (PR) is  $0.32 \pm 0.19$ . All the parameters of semen were abnormal distribution.

The total sperm number is  $148.92 \pm 123.43 \times 10^6$  per ejaculate in the year of 2018,  $146.21 \pm 122.75 \times 10^6$  per ejaculate in the year of 2019,  $144.29 \pm 110.72 \times$

$10^6$  per ejaculate in the year of 2020 to Feb. 2021. Sperm concentration is  $43.74 \pm 29.97 \times 10^6$  per ml in 2018,  $41.67 \pm 28.48 \times 10^6$  per ml in 2019,  $41.32 \pm 26.44 \times 10^6$  per ml in 2010 to Feb. 2021. There was a significant decreasing trend in the concentration ( $P < 0.01$ ). On the contrary, the PR and total PR number had been increased. The PR for three years were  $0.28 \pm 0.18$ ,  $0.33 \pm 0.19$  and  $0.34 \pm 0.19$  respectively, and the total PR number were  $50.64 \pm 61.99 \times 10^6$  per ejaculate,  $56.13 \pm 64.20 \times 10^6$  per ejaculate and  $56.42 \pm 59.89 \times 10^6$  per ejaculate respectively ( $p < 0.01$ ).

All the data were divided into four groups according to seasons. The results of each group are shown in **Table 2**. According to the data, semen volume was the highest in winter ( $3.63 \pm 1.61$  ml,  $p < 0.05$ ), the sperm concentration, total sperm number and total PR number were more in winter and spring than in summer and autumn ( $p < 0.01$ ), and the total motility and PR were the lowest in summer ( $0.50 \pm 0.22$ ,  $0.31 \pm 0.19$ ;  $p < 0.01$ ).

**Table 1.** Basic data on patient's semen.

YEAR	Total	2018	2019	2020-2021.2	P
Number	21,715	8029	7502	6184	
Age (year)	$31.19 \pm 5.58$	$31.36 \pm 5.74$	$31.07 \pm 5.47$	$31.10 \pm 5.49$	0.074
Abstinence days (day)	$4.06 \pm 1.22$	$4.00 \pm 1.20$	$4.18 \pm 1.22$	$4.00 \pm 1.24$	<0.01
Semen volume (ml)	$3.57 \pm 1.61$	$3.52 \pm 1.60$	$3.61 \pm 1.66$	$3.59 \pm 1.54$	<0.01
Sperm concentration ( $10^6$ per ml)	$42.34 \pm 28.50$	$43.74 \pm 29.97$	$41.67 \pm 28.48$	$41.32 \pm 26.44$	<0.01
Total sperm number ( $10^6$ per ejaculate)	$146.67 \pm 119.72$	$148.92 \pm 123.43$	$146.21 \pm 122.75$	$144.29 \pm 110.72$	0.263
Progressive motility (PR)	$0.32 \pm 0.19$	$0.28 \pm 0.18$	$0.33 \pm 0.19$	$0.34 \pm 0.19$	<0.01
Total PR number ( $10^6$ per ejaculate)	$54.18 \pm 62.23$	$50.64 \pm 61.99$	$56.13 \pm 64.20$	$56.42 \pm 59.89$	<0.01
Total motility (PR + NP)	$0.51 \pm 0.22$	$0.51 \pm 0.22$	$0.51 \pm 0.22$	$0.53 \pm 0.21$	<0.01
PH	$7.39 \pm 0.16$	$7.39 \pm 0.17$	$7.40 \pm 0.15$	$7.38 \pm 0.15$	<0.01

**Table 2.** Semen data grouped by season.

Number	Spring	Summer	Fall	Winter	P
	5106	5961	5535	5113	
Age (year)	$31.29 \pm 5.59$	$31.00 \pm 5.62$	$31.31 \pm 5.52$	$31.16 \pm 5.57$	0.010
Semen volume (ml)	$3.56 \pm 1.65$	$3.56 \pm 1.59$	$3.54 \pm 1.58^*$	$3.63 \pm 1.61^{**}$	0.008
Sperm concentration ( $10^6$ per ml)	$43.98 \pm 30.03^{**}$	$41.63 \pm 29.10$	$40.58 \pm 27.13$	$43.43 \pm 27.54$	<0.01
Total sperm number ( $10^6$ per ejaculate)	$151.70 \pm 125.07$	$143.19 \pm 118.70$	$139.11 \pm 111.94^*$	$153.87 \pm 122.93^{**}$	<0.01
Progressive motility (PR)	$0.32 \pm 0.19$	$0.31 \pm 0.19^*$	$0.32 \pm 0.19$	$0.32 \pm 0.19$	<0.01
Total PR number ( $10^6$ per ejaculate)	$57.29 \pm 66.12^{**}$	$51.96 \pm 61.63^*$	$52.08 \pm 58.58$	$55.96 \pm 62.61$	<0.01
Total motility (PR + NP)	$0.52 \pm 0.22$	$0.50 \pm 0.22^*$	$0.52 \pm 0.21$	$0.52 \pm 0.21$	<0.01
PH	$7.40 \pm 0.16$	$7.39 \pm 0.16$	$7.40 \pm 0.17$	$7.38 \pm 0.16$	<0.01

\*\* : maximum value; \* : minimum value.

### 3.2. Meteorological Data

All the meteorological parameters are abnormal distribution. There is no statistical difference in the mean value of various parameters of meteorological data from the year of 2018 to 2020 (the data are not listed). According to these data, average air pressure, minimum pressure and maximum pressure are all the highest in winter ( $1012.27 \pm 3.70$  hPa,  $1009.42 \pm 3.67$  hPa,  $1014.45 \pm 3.87$  hPa), and the lowest in summer ( $996.78 \pm 2.88$  hPa,  $994.62 \pm 2.93$  hPa,  $998.49 \pm 2.91$  hPa). Average temperature, minimum temperature and maximum temperature are the lowest in winter ( $15.08^\circ\text{C} \pm 3.74^\circ\text{C}$ ,  $11.73^\circ\text{C} \pm 4.09^\circ\text{C}$ ,  $21.00^\circ\text{C} \pm 4.57^\circ\text{C}$ ) and the highest in summer ( $28.42^\circ\text{C} \pm 1.70^\circ\text{C}$ ,  $25.35^\circ\text{C} \pm 1.31^\circ\text{C}$ ,  $33.09^\circ\text{C} \pm 2.50^\circ\text{C}$ ). In terms of air humidity, it is lowest in winter ( $74.72\% \pm 12.89\%$ ,  $51.42\% \pm 17.03\%$ ) and highest in spring ( $83.87\% \pm 8.14\%$ ,  $62.99\% \pm 16.01\%$ ). The isolation duration is the longest in autumn ( $5.94 \pm 3.72$  hour) and shortest in spring ( $3.07 \pm 3.56$  hour). All *p* values were less than 0.05 (**Table 3**).

### 3.3. Correlation Analysis

The correlation analysis of semen parameters showed that there was a strong positive correlation between total motility and progressive motility (PR) ( $r = 0.934$ ,  $p < 0.01$ ). Total sperm number was positively correlated with sperm concentration and total PR number respectively ( $r = 0.826$ ,  $p < 0.01$ ;  $r = 0.857$ ,  $p < 0.01$ ) and weakly correlated with total motility and PR ( $r = 0.508$ ,  $p < 0.01$ ;  $r = 0.420$ ,  $p < 0.01$ ). There was a strong correlation between semen concentration and total PR number ( $r = 0.755$ ,  $p < 0.01$ ), but a weak correlation with total motility and PR ( $r = 0.575$ ,  $p < 0.01$ ;  $r = 0.440$ ,  $p < 0.01$ ). The total PR number was positively correlated with total motility and PR ( $r = 0.828$ ,  $p < 0.01$ ;  $r = 0.803$ ,  $p < 0.01$ ). The amount of ejaculation was weakly correlated with the total sperm number ( $r = 0.423$ ,  $p < 0.01$ ), and had no correlation with other parameters (**Table 4**).

Correlation analysis between semen data and meteorological parameters was performed. The weather data were moved forward for 72 days and matched with semen analysis data. There was no correlation between insolation duration and semen parameters. Semen volume, sperm total motility and PR had no correlated with meteorological data. On the correlation analysis of other participants, total sperm number, sperm concentration and total PR number were positively correlated with atmospheric pressure ( $p < 0.01$ ) and negatively correlated with air temperature ( $p < 0.01$ ), total sperm number was negatively correlated with air humidity and the semen volume was only positively correlated with the minimum pressure ( $p < 0.05$ ). The relationship between sperm concentration and air average pressure and air average temperature on and before 72 days receiving specimens were showed in **Figure 1**.

## 4. Discussion

The aim of this study was to investigate the relationship between sperm parameters and environmental factors.

**Table 3.** Meteorological data grouped by season.

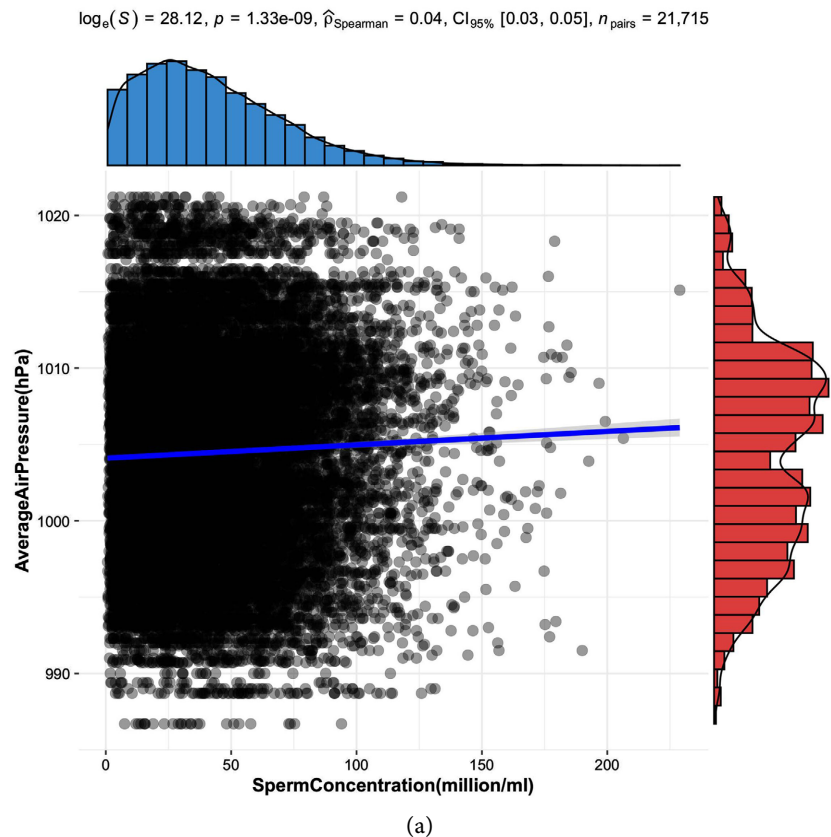
Season	Spring	Summer	Autumn	Winter	Total	p
Average Air pressure (hPa)	1005.13 ± 4.46*	996.78 ± 2.88	1005.84 ± 4.50*	1012.27 ± 3.70	1005.55 ± 6.72	<0.01
Minimum pressure (hPa)	1002.34 ± 3.97	994.62 ± 2.93	1003.62 ± 4.66	1009.42 ± 3.67	1003.04 ± 6.50	<0.01
Maximum pressure (hPa)	1006.99 ± 4.23*	998.49 ± 2.91	1007.99 ± 4.61*	1014.45 ± 3.87	1007.55 ± 6.87	<0.01
Average temperature (°C)	22.50 ± 4.41*	28.42 ± 1.70	23.54 ± 3.69*	15.08 ± 3.74	21.98 ± 5.99	<0.01
Minimum temperature (°C)	19.53 ± 4.42*	25.35 ± 1.31	20.21 ± 3.86*	11.73 ± 4.09	18.77 ± 6.14	<0.01
Maximum temperature (°C)	27.01 ± 4.56	33.09 ± 2.50	29.08 ± 4.12	21.00 ± 4.57	27.21 ± 6.00	<0.01
Mean relative humidity (%)	83.87 ± 8.14**	83.48 ± 7.65**	79.08 ± 10.86	74.72 ± 12.89	79.83 ± 11.01	<0.01
Minimum relative humidity (%)	62.99 ± 16.01**	61.00 ± 11.69**	54.15 ± 13.35***	51.42 ± 17.03***	56.76 ± 15.48	<0.01
Insolation duration (h)	3.07 ± 3.56	5.07 ± 3.37	5.94 ± 3.72	4.23 ± 3.83	4.65 ± 3.79	<0.01

\* p > 0.05 in spring and autumn; \*\* p > 0.05 in spring and summer; \*\*\* p > 0.05 in autumn and winter.

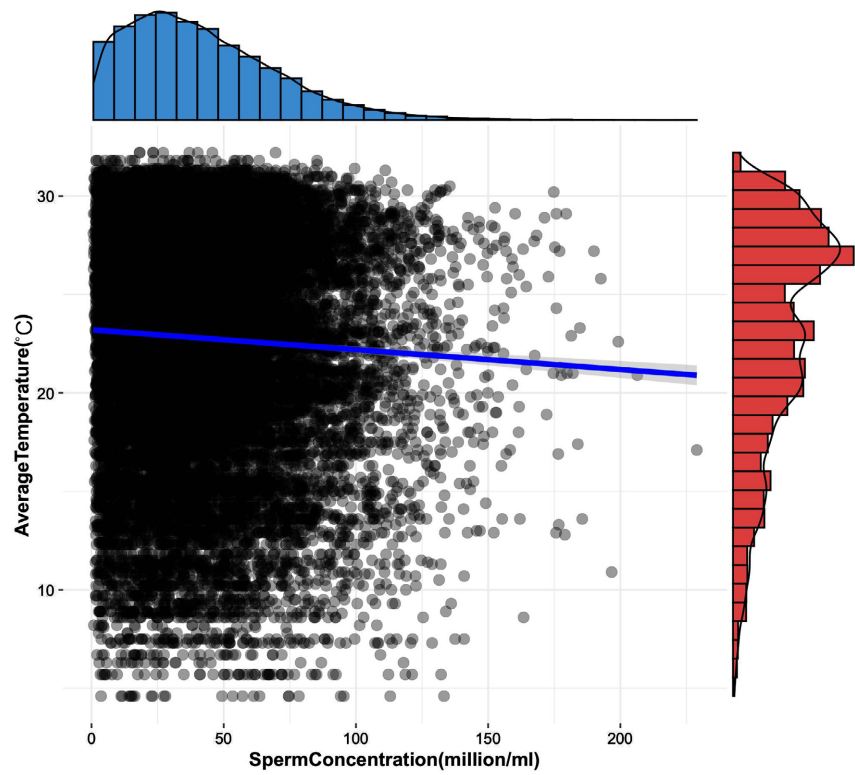
**Table 4.** Relationship between semen parameters.

	Semen volume (ml)	Sperm concentration (10 <sup>6</sup> per ml)	Total motility (PR + NP)	Progressive motility (PR)	Total sperm number (10 <sup>6</sup> per ejaculate)
Sperm concentration (10 <sup>6</sup> per ml)	-0.092				
Total motility (PR + NP)	-0.015*	0.575			
Progressive motility (PR)	0.033	0.440	0.934		
Total sperm number (10 <sup>6</sup> per ejaculate)	0.423	0.826	0.508	0.420	
Total PR number (10 <sup>6</sup> per ejaculate)	-0.296	0.755	0.828	0.803	0.857

\* p > 0.05.

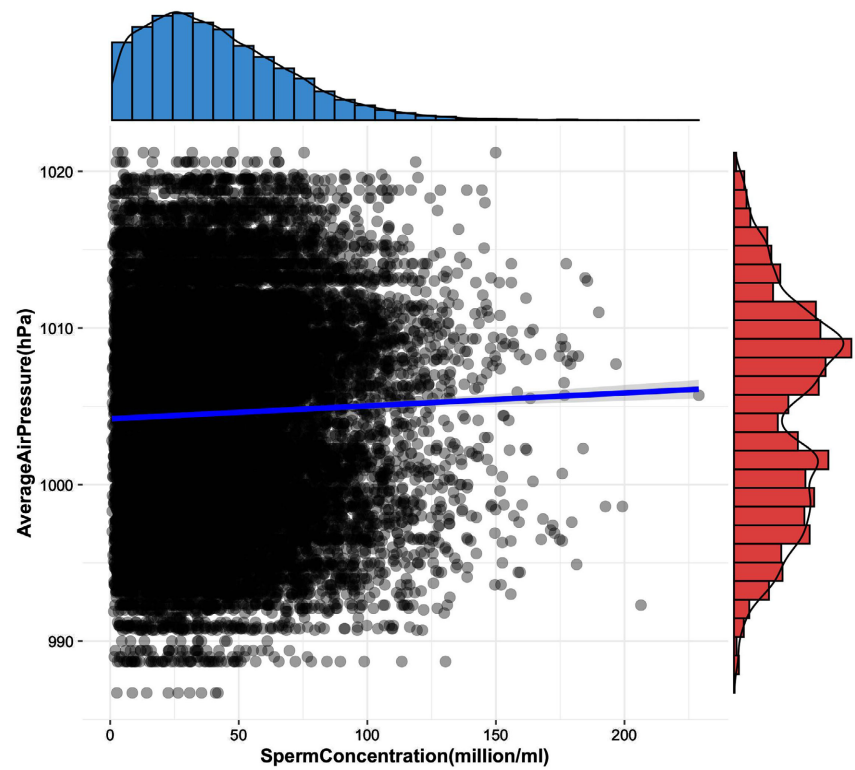


$\log_e(S) = 28.21, p = 2.07e-12, \hat{\rho}_{\text{Spearman}} = -0.05, \text{CI}_{95\%} [-0.06, -0.03], n_{\text{pairs}} = 21,715$



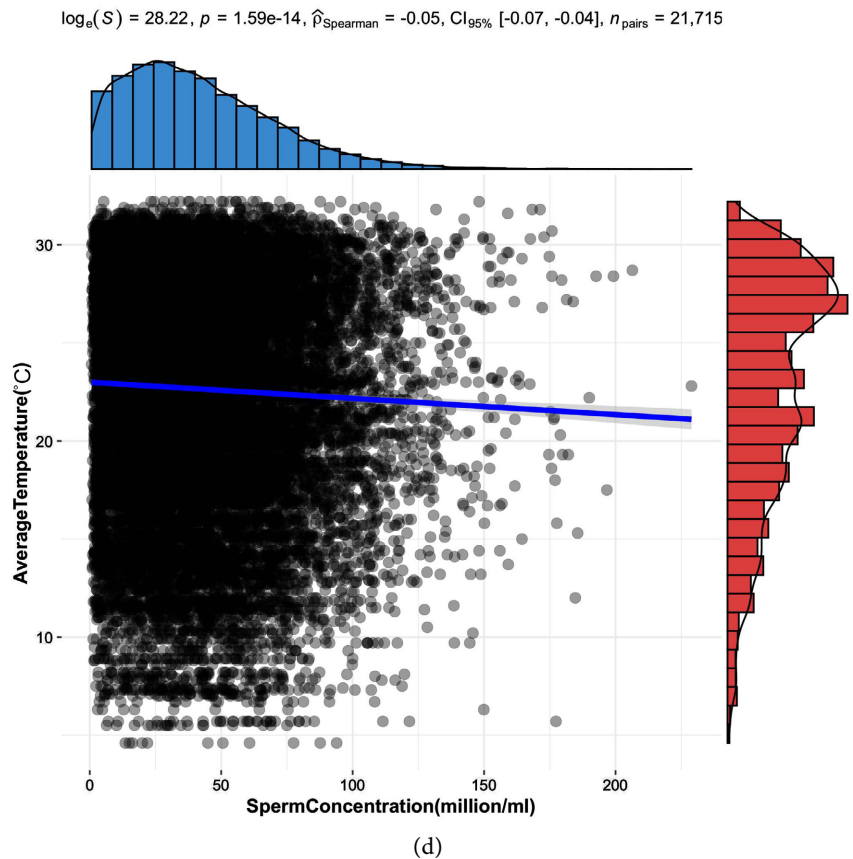
(b)

$\log_e(S) = 28.12, p = 1.74e-09, \hat{\rho}_{\text{Spearman}} = 0.04, \text{CI}_{95\%} [0.03, 0.05], n_{\text{pairs}} = 21,715$



(c)





**Figure 1.** Relationship between Sperm Concentration and Average Air Pressure and Average Temperature. (a) and (b): Relationship between Sperm Concentration and Average Air Pressure and Average Temperature 72 days before receiving specimens. (c) and (d): The relationship between Sperm Concentration and Average Air Pressure and Average Temperature on the day of receiving specimen.

Previous studies have shown that sperm concentrations have been declining in recent decades, with the average human sperm concentration dropping by about 50 percent over the past 50 years, from  $113 \times 10^6/\text{ml}$  to  $61 \times 10^6/\text{ml}$  [12]. This trend is similar to our study. The average semen concentration was  $42.34 \pm 28.50 \times 10^6/\text{ml}$ . The semen concentration declined from  $43.74 \pm 29.97$  per ml in the year of 2018 to  $41.32 \pm 26.44$  per ml in the year of 2020, which was much lower than previous studies. Although sperm concentration decreased, semen volume and the proportion of PR increased, resulting in an increase in the total number of PR. Total number of sperm decreased, but there was no significant statistical significance. There were other factors in and out of the body that affecting male fertility, so no firm conclusions can be drawn from semen analysis alone. According to the records of the national meteorological station obtained from Guangzhou Climate Center, the annual average temperature, air pressure and air humidity in Guangzhou had no significant variations from 2018 to 2020. Therefore, the trend of decreasing or increasing among parameters may also be related to other factors, such as environmental pollution, mental stress, and extracellular environment of spermatogenesis [20]. But the timespan is only three years, more



data are needed.

In our study, there was a strong positive correlation between sperm motility and PR ( $r = 0.934$ ,  $p = 0.000$ ). Total sperm number was positively correlated with sperm concentration and total PR ( $r = 0.826$ ,  $p = 0.000$ ;  $r = 0.857$ ,  $p = 0.000$ ). There was a strong correlation between semen concentration and total PR number ( $r = 0.755$ ,  $p = 0.000$ ), which indicate that semen concentration and total sperm number may better reflect semen quality than other parameters. In the evaluation of male pregnancy rate, semen concentration and total sperm number can be highly concerned parameters in clinic.

The climate factor itself varies with the season, with both temperature and humidity triggering physiological responses that regulate body temperature. In recent years, several studies have addressed the possible relationship between rising temperatures and the ability of human reproduce [21] [22]. Other factors of climate, such as atmospheric pressure and exposure to sunlight, may also affect sperm quality [23]. The latter affects reproduction directly through endocrine regulation, but also indirectly by influencing solar radiation [16]. All of these factors should be considered when discussing the overall impact of climate on sperm quality.

Guangdong has a subtropical monsoon climate, spring is warm and rainy, summer is hot and humid, winter is mild and dry, the four seasons are relatively distinct [18] [19]. Our study found that there were seasonal differences in sperm quality, and the sperm quality was relatively high in winter and spring, which was consistent with the study of Levitas *et al.* who assessed 6455 semen samples and found that the sperm quantity and quality were higher in winter and spring [24]. Seasonal changes in chromatin condensation in human sperm seem to confirm this hypothesis [25]. Studies in Italy have also shown that semen changes may follow a seasonal pattern, but it needs at least two years of data to support [26]. Our study collected semen analysis data for three years, with more than 20,000 samples, which can reflect the relationship between sample parameters and seasons.

Although the data support the view that sperm parameters change seasonally in normal and oligospermic sperm samples, no relationship was found between the seasonal patterns of sperm parameters and human pregnancy [13]. Therefore, although there were better sperm parameters in both spring and winter, further research is needed to determine whether higher treatment success rates will be achieved for infertile patients during these seasons.

In humans, the effect of seasonal rotation on sperm parameters is still controversial, and some studies have not formalized the effect of season on sperm parameters [27]. It has been suggested that sperm quality parameters are more likely to be related to climatic factors measured two months before semen collection, rather than in the same month [13]. Therefore, we moved the weather data forward 72 days and matched it with the semen analysis data. We speculated that climate factors played a certain role in the spermatogenesis and sperm

development, although it did not provide enough information statistically. However, the influence of temperature, humidity and air pressure on sperm parameters was still objective ( $p < 0.01$ ). The higher the temperature, the higher the humidity, the poorer the sperm quality. Therefore, in the clinical analysis of semen results, it is necessary to consider the factors of current season and comprehensively evaluate the semen results.

In terms of insolation duration, animal model studies suggested that seasonal variations may be mediated by endogenous mechanisms related to daylight length [28]. Our data showed that insolation duration had no significant effect on any of the parameters of semen analysis, which is similar to the results of a study in Turkey [29]. It is possible that the effects of sunlight on the change of human semen parameters may be relatively slow, which requires long-term observation and comparison, such as decades or even hundreds of years, or the data of regions with different insolation duration.

Our study indicates that semen quality might be affected by season and related to temperature, humidity and atmospheric pressure suggesting that in addition to the use of food supplement and medication [30], changes in these three factors may improve semen quality, and fertility guidance may also vary by season. Studies have determined that any factor that may increase testicular temperature, such as cryptorchidism, outdoor high-temperature operations, and saunas will have an adverse impact on spermatogenesis [31] [32]. It is recommended that the patient may take appropriate measures to reduce testicular temperature to improve sperm quality such as avoiding study and work in overheated environment, wearing boxer shorts [33]. There are few studies on the effects of humidity and pressure around testis on sperm quality, but together with temperature, they are a group of inseparable indicators, best testicular environmental indicators might be found in following studies.

Unfortunately, the semen analysis data in February 2020 were lacked due to COVID-19. In addition, we only discussed the results of routine semen analysis. Post-testicular factors also play a significant role in sperm fertilization, including the ability to acquire motility, recognize and bind zona pellucida, and fuse with oocytes. These different properties are acquired gradually and simultaneously as sperm pass through the epididymis. Therefore, sperm morphology, membrane function and spermatoplasm environment may also be affected by the external environment, which together play an important role in spermatogenesis maturation.

## 5. Conclusion

Finally, seasonal factors should be considered when evaluating male reproductive ability. Except for considering conventional semen analysis parameters, other factors should also be comprehensively analyzed, and adjust the treatment and intervention measures to improve semen quality and fertility according to the actual situation of the environment.

## Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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