Environmental Research 236 (2023) 116665

Contents lists available at ScienceDirect

Environmental Research

journal homepage: www.elsevier.com/locate/envres

# Heat wave exposure and semen quality in sperm donation volunteers: A retrospective longitudinal study in south China

Xinyi Deng [a,1], Qiling Wang [b,1], Chunxiang Shi [c], Jing Wei [d], Ziquan Lv [e], Suli Huang [f], Yong-Gang Duan [g], Xinzong Zhang [b,**], Yuewei Liu [a,*]

[a] Department of Epidemiology, School of Public Health, Sun Yat-sen University, Guangzhou, Guangdong, 510080, China
[b] NHC Key Laboratory of Male Reproduction and Genetics, Guangdong Provincial Reproductive Science Institute, Guangdong Provincial Fertility Hospital, Guangzhou, Guangdong, 510600, China
[c] Meteorological Data Laboratory, National Meteorological Information Center, Beijing, 100081, China
[d] Department of Atmospheric and Oceanic Science, Earth System Science Interdisciplinary Center, University of Maryland, College Park, MD, 20740, USA
[e] Central Laboratory of Shenzhen Center for Disease Control and Prevention, Shenzhen, Guangdong, 518055, China
[f] Department of Environment and Health, Shenzhen Center for Disease Control and Prevention, Shenzhen, Guangdong, 518055, China
[g] Shenzhen Key Laboratory of Fertility Regulation, Centre of Assisted Reproduction and Embryology, The University of Hong Kong-Shenzhen Hospital, Shenzhen, Guangdong, 518053, China

## ARTICLE INFO

Handling Editor: Jose L Domingo

*Keywords:*
Heat wave
Semen quality
Windows of susceptibility
Longitudinal study
Linear mixed model
Distributed lag non-linear model

## ABSTRACT

*Background:* Previous studies have suggested an association between non-optimum ambient temperature and decreased semen quality. However, the effect of exposure to heat waves on semen quality remains unclear.

*Methods:* Volunteers who intended to donate sperm in Guangdong provincial human sperm bank enrolled. Heat waves were defined by temperature threshold and duration, with a total of 9 definitions were employed, specifying daily mean temperature exceeding the 85th, 90th, or 95th percentile for at least 2, 3, or 4 consecutive days. Residential exposure to heat waves during 0–90 days before ejaculation was evaluated using a validated gridded dataset on ambient temperature. Association and potential windows of susceptibility were evaluated and identified using linear mixed models and distributed lag non-linear models.

*Results:* A total of 2183 sperm donation volunteers underwent 8632 semen analyses from 2018 to 2019. Exposure to heat wave defined as daily mean temperature exceeding the 95th percentile for at least 4 consecutive days (P95–D4) was significantly associated with a 0.11 (95% confidence interval [CI]: 0.03, 0.18) ml, 3.36 (1.35, 5.38) × $10^6$/ml, 16.93 (7.95, 25.91) × $10^6$, and 2.11% (1.4%, 2.83%) reduction in semen volume, sperm concentration, total sperm number, and normal forms, respectively; whereas exposure to heat wave defined as P90–D4 was significantly associated with a 1.98% (1.47%, 2.48%) and 2.08% (1.57%, 2.58%) reduction in total motility and progressive motility, respectively. Sperm count and morphology were susceptible to heat wave exposure during the early stage of spermatogenesis, while sperm motility was susceptible to exposure during the late stage.

*Conclusion:* Heat wave exposure was significantly associated with a reduction in semen quality. The windows of susceptibility during 0–90 days before ejaculation varied across sperm count, motility, and morphology. Our findings suggest that reducing heat wave exposure before ejaculation may benefit sperm donation volunteers and those attempting to conceive.

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* Corresponding author. Department of Epidemiology, School of Public Health, Sun Yat-sen University, 74 Zhongshan Second Road, Guangzhou, Guangdong, 510080, China.

** Corresponding author. NHC Key Laboratory of Male Reproduction and Genetics, Guangdong Provincial Reproductive Science Institute, Guangdong Provincial Fertility Hospital. 17 Meidong Road, Guangzhou, Guangdong, 510600, China.

*E-mail addresses:* 13857170787@139.com (X. Zhang), liuyuewei@mail.sysu.edu.cn (Y. Liu).

[1] These authors contributed equally to this work.

https://doi.org/10.1016/j.envres.2023.116665
Received 10 May 2023; Received in revised form 23 June 2023; Accepted 12 July 2023

## 1. Introduction

Semen quality is critical to male fertility potential (Bartoov et al., 1993; Bonde et al., 1998; Zinaman et al., 2000). In the field of reproductive health, concerns have been raised about a global decline in semen quality over the past few decades as considerable evidence has accumulated to suggest (Carlsen et al., 1992; Lv et al., 2021; Virtanen et al., 2017). Previous studies have linked exposure to non-optimum ambient temperature exposure to the reduction in semen quality. For instance, occupational studies have identified a higher risk of declined semen quality for workers with heat exposure including welders, bakers, and ironworkers (Bonde, 1992; Hamerezaee et al., 2018; Thonneau et al., 1998); several studies in sperm banks or fertility clinics have reported a significant seasonal variation, with a study in New Orleans finding lower sperm concentration, total sperm per ejaculate, percent motile sperm, and motile sperm concentration during the summer (Levine et al., 1988), and a study in Zhejiang, China finding lower semen volume, sperm concentration, and normal morphology during midsummer with average highest temperature higher than 30 °C (Zhang et al., 2013). In our previous study in Wuhan, China, we observed inverted U-shaped exposure-response relationships of ambient temperature with sperm count and motility among sperm donation volunteers, suggesting that exposure to both lower and higher ambient temperature contributes to a reduction in semen quality (Zhou et al., 2020). Nevertheless, the impact of heat wave, of which the nature is related to non-optimum temperature, on semen quality remains poorly understood. Heat wave is a type of climatic hazard characterized by periods of prolonged unusually high temperatures, often defined with reference to a relative temperature threshold, lasting for a specific time period (IPCC et al., 2022). Exposure to heat waves has been associated with a variety of adverse outcomes in reproductive health, such as preterm birth and low birth weight (Zhang et al., 2017). Given the temperature-related nature of heat wave and the susceptibility of semen quality to non-optimum temperature, it is plausible to make the presumption that heat waves contribute to the decline of semen quality.

Spermatogenesis is a complex process whereby spermatozoa develop from the primordial germ cells. This process can be divided into three phases: (1) spermatocytogenesis, a process of proliferation and differentiation of spermatogonia, (2) meiosis, and (3) spermiogenesis, a process of transformation of round spermatids after meiosis into spermatozoa (Sharma and Agarwal, 2011). Given the time required for spermatogenesis, a maximum lag of 90 days for exposure assessment is suggested by much of the literature, within which potential windows of susceptibility to exposure may play a key role (Johnson et al., 2010). Information on the windows of susceptibility can be useful in exploring biological mechanisms as well as in the implementation of specific interventions. Nevertheless, as a common approach adopted in previous studies, using a mean temperature to characterize individual exposure on a relatively long timescale (i.e., a 90-day period before semen collection) rendered difficulties in the identification of the potential windows of susceptibility in the process of spermatogenesis.

In the context of the ongoing climate change and growing population density due to rapid urbanization, the frequency and intensity of heat waves are expected to escalate, as is the number of reproductive-aged males exposed to them (IPCC et al., 2013; Watts et al., 2015). To improve the understanding of potential adverse effects of heat waves on male reproductive health, this longitudinal study among sperm donation volunteers in Guangdong province, China aimed to explore the association of exposure to heat waves with semen quality, and to identify its potential windows of susceptibility.

## 2. Materials and methods

### 2.1. Study design and participants

In this retrospective longitudinal study, sperm donation volunteers who lived in Guangdong, China and intended to donate sperm at the Guangdong provincial human sperm bank between June 22, 2018 and December 31, 2019 were included. Volunteers were screened to determine their qualification for donation according to the standard guidelines published by the Ministry of Health of the People's Republic of China (Ministry of Health of the People's Republic of China, 2003), and volunteers with conditions such as varicocele, orchiditis, and epididymitis, which may affect semen quality, were not considered as potential sperm donors. After complying with the criteria of primary screening, all volunteers underwent further laboratory tests, including at least one semen analysis. Qualified donors were generally instructed to donate sperm multiple times and tested for semen quality before each donation. After the exclusion of 299 volunteers not eligible for 2–7 days of abstinence and 119 volunteers without completed information of either demographic characteristics or exposure, 2183 volunteers who underwent 8653 semen analyses were finally included in the study (Supplementary Fig. S1). The study was approved by the Ethics Committee of the School of Public Health, Sun Yat-sen University with a waiver of informed consent.

### 2.2. Semen analysis

The study outcome of interest was semen quality, which was determined using semen parameters including semen volume (ml), sperm concentration ($10^6$/ml), total sperm number ($10^6$), total motility (%), progressive motility (%), and normal forms (%). Semen analysis was conducted following the World Health Organization (WHO) laboratory manual, as has been described in detail elsewhere (World Health Organization, 2010; Yang et al., 2021; Zhang et al., 2023). Refer to the Supplementary File S1 for detailed process of semen analysis.

### 2.3. Exposure assessment

To account for potential acclimatization to local temperature patterns (Guo et al., 2017), a heat wave was defined as the daily temperature exceeding a threshold in a given grid (i.e., the 85th, 90th and 95th percentile based on the distribution of daily temperatures during 2018–2019 in this grid) for a certain number of consecutive days (i.e., at least 2, 3, or 4 days) (Robinson, 2001). Accordingly, from P85–D2 (at least 2 consecutive days with the daily temperature exceeding the 85th percentile) to P95−D4 (at least 4 consecutive days with the daily temperature exceeding the 95th percentile), a total of 9 heat wave definitions were specified (see Supplementary Table S1 for detailed description for each definition). To determine the residential based temperature threshold, gridded daily 24-h average temperature data with a spatial resolution of $0.0625^\circ \times 0.0625^\circ$ for Guangdong province covering 2018–2019 were obtained from the China Meteorological Administration Land Data Assimilation System (CLDAS V-2.0) (Han et al., 2020; Liu et al., 2019; Tie et al., 2022). This dataset provides high accuracy with a bias of 0.15 °C and a root mean square error (RMSE) of 0.68 °C in Guangdong province. We extracted the heat wave exposure history for each of the 90 days before the date of semen collection at each participant's geocoded residential address.

### 2.4. Demographic information, semen collection information, and other environmental factors

Demographic characteristics of each participant and information on each semen collection were retrieved from the electronic information registration system of the Guangdong provincial human sperm bank. Demographic characteristics include age, ethnicity, educational attainment, marital status, fertility status (ever having fathered any biological child), and body mass index (BMI). BMI was calculated as weight (kg) divided by height (m) squared, and categorized into underweight ($<18.5$ kg/m$^2$), normal weight (18.5–23.9 kg/m$^2$), overweight (24.0–27.9 kg/m$^2$), and obese ($\geq 28.0$ kg/m$^2$) according to the recommended criteria for the Chinese people (Zhou, 2002). Information on semen collection includes abstinence period, year at semen collection, and season at semen collection.

Other environmental factors that could be potential confounders include ambient temperature, relative humidity, and fine particulate matter ($PM_{2.5}$). Gridded daily 24-h average $PM_{2.5}$ concentration data with a 1 km × 1 km spatial resolution were obtained from the ChinaHighAirPollutants (CHAP) dataset (Wei and Li, 2019; Wei et al., 2020, 2021). This dataset yields a high quality with a cross-validation coefficient of determination ($R^2$) of 0.92 and a root mean square error (RMSE) of 10.76 $\mu g/m^3$ on a daily basis. Using the CLDAS and CHAP dataset, we extracted daily 24-h average temperature, relative humidity, and $PM_{2.5}$ concentration during each of the 90 days before the date of semen collection at each participant's residential address, and assessed their exposure by calculating a 90-day average for temperature, relative humidity, and $PM_{2.5}$ concentration.

### 2.5. Statistical analyses

Linear mixed models (LMM) with subject-specific random intercepts were used to estimate the association of exposure to heat waves under different definitions with all semen parameters except for normal forms. For normal forms, which were only measured once regardless of whether a participant underwent multiple semen collections and analyses, linear models (LM) for single measure data were applied. Changes (95% confidence interval [CI]) in semen parameters associated with exposure to heat waves were estimated with adjustment for age (20–25, 26–30, or 31–45 years), educational attainment (under college, college and higher), marital status (unmarried, married, divorced), ever having fathered any biological child (yes, no), year at semen collection (2018, 2019), season at semen collection (Mar–May as spring, Jun–Aug as summer, Sep–Nov as autumn, Dec and Jan–Feb as winter), temperature (natural cubic spline with 3 degrees of freedom [*df*]), relative humidity (natural cubic spline with 3 *df*), and $PM_{2.5}$ concentration (natural cubic spline with 3 *df*). A directed acyclic graph (DAG) was employed to guide the selection of covariates (Supplementary Fig. S7).

To further identify potential windows of susceptibility to heat wave exposures for semen quality, a cross-basis function constructed with distributed lag non-linear model (DLNM) was introduced in the LMM or LM (Gasparrini, 2014). The cross-basis function for heat wave exposure was a bi-dimensional function defined with a linear exposure-response function and a natural cubic spline with 4 *df* for the lag structure. The formulas of all the models are shown in detail in the Supplementary File S2. Changes (95% CIs) in each semen parameter with heat wave exposure at different lag days were estimated and plotted to present the lag-response associations of heat wave exposure with semen quality. Days with significant effects were considered as the potential windows of susceptibility for the specific semen parameter.

A sensitivity analysis was conducted to include only those participants who resided in Guangzhou, comprising 82.5% of the total participants. We also divided the 90-day period into four exposure windows: (1) 0–9 days, (2) 10–14 days, (3) 15–69 days, and (4) 70–90 days before semen collection, which is consistent with the empirical division used in most previous studies to explore windows of susceptibility (Chen et al., 2021; Wu et al., 2022; Yang et al., 2021). To assess the association between exposure to heat waves in the specific exposure windows and semen quality, all four exposure windows were included simultaneously in the same model. Further sensitivity analyses conducted included adjusting for age as a continuous variable, excluding participants aged over 30, including participants with illegible abstinence periods, and categorizing the exposure to heat waves under different definitions based on the number of exposures. Z-test was employed to compare the estimates from the sensitivity analysis models and the main models.

All statistical analyses were performed in R (version 4.3.0) using the *lme4* and *dlnm* packages (Bates et al., 2015; Gasparrini, 2011; R Core Team, 2023). All tests were two sided with a level of significance set at $p < 0.05$.

## 3. Results

### 3.1. Study participants and characteristics

During the study period, the 2183 volunteers underwent a total of 8632 semen analyses. Of these volunteers, 815, 376, 141, and 76 provided one, two, three, and four semen samples, respectively. The mean number of semen collections per participant was 3.95. Most participants were Han Chinese (96.9%), college-educated or higher (84.4%), and unmarried (79.8%). The mean age and BMI were 26.2 years and 22.3 $kg/m^2$, respectively (Table 1). Fig. 1 shows the spatial distribution of the count occurrences of heat wave, mean temperature, and the residential addresses of the participants in Guangdong Province, China during 2018–2019. From the mildest definition P85–D2 to the most extreme definition P95−D4, the count occurrences of heat wave decreased gradually, with the most extreme heat waves and highest mean temperature typically occurring in the southwest of Guangdong, closer to the equator (Fig. 1A and B). Participants were recruited from various cities within Guangdong province, with the majority residing in Guangzhou (Fig. 1C). The distribution of semen parameters, meteorological variables and $PM_{2.5}$ concentration during the 90 days before semen collection for each semen analysis is presented in Table 2. Under the P85–D2 definition, about 73% of the 8632 semen analyses were exposed to heat waves at least once during the 90 days before the date of semen collection (Supplementary Table S2). The mean squares within (MSW), within-subject coefficient of variation ($CV_w$), mean squares between (MSB), between-subject coefficients of variation ($CV_b$), and

**Table 1**
Characteristics of the participants and semen analyses.

| Characteristic | N | Mean ± SD or % |
|---|---|---|
| Number of participants | 2183 | |
| Age, years | | 26.2 ± 5.4 |
| 20–25 | 1129 | 51.7 |
| 26–30 | 561 | 25.7 |
| 31–45 | 493 | 22.6 |
| Ethnicity | | |
| Han | 2116 | 96.9 |
| Other | 67 | 3.1 |
| Educational attainment | | |
| College or higher | 1842 | 84.4 |
| Under college | 341 | 15.6 |
| Marital status | | |
| Unmarried | 1741 | 79.8 |
| Married | 405 | 18.6 |
| Divorced | 37 | 1.7 |
| Ever having fathered any biological child | | |
| No | 1835 | 84.1 |
| Yes | 348 | 15.9 |
| BMI ($kg/m^2$) | | 22.3 ± 3.3 |
| <18.5 | 239 | 10.9 |
| 18.5–23.9 | 1379 | 63.2 |
| 24.0–27.9 | 440 | 20.2 |
| >28.0 | 125 | 5.7 |
| Number of semen analyses | 8632 | |
| Abstinence period before semen collection, days | | 4.3 ± 1.2 |
| 2–3 | 2614 | 30.3 |
| 4–5 | 4742 | 54.9 |
| 6–7 | 1276 | 14.8 |
| Year at semen collection | | |
| 2018 | 2359 | 27.3 |
| 2019 | 6273 | 72.7 |
| Season at semen collection | | |
| Spring (Mar–May) | 1883 | 21.8 |
| Summer (Jun–Aug) | 2458 | 28.5 |
| Autumn (Sep–Nov) | 2571 | 29.8 |
| Winter (Dec, Jan, Feb) | 1720 | 19.9 |

BMI = body mass index; SD = Standard deviation.

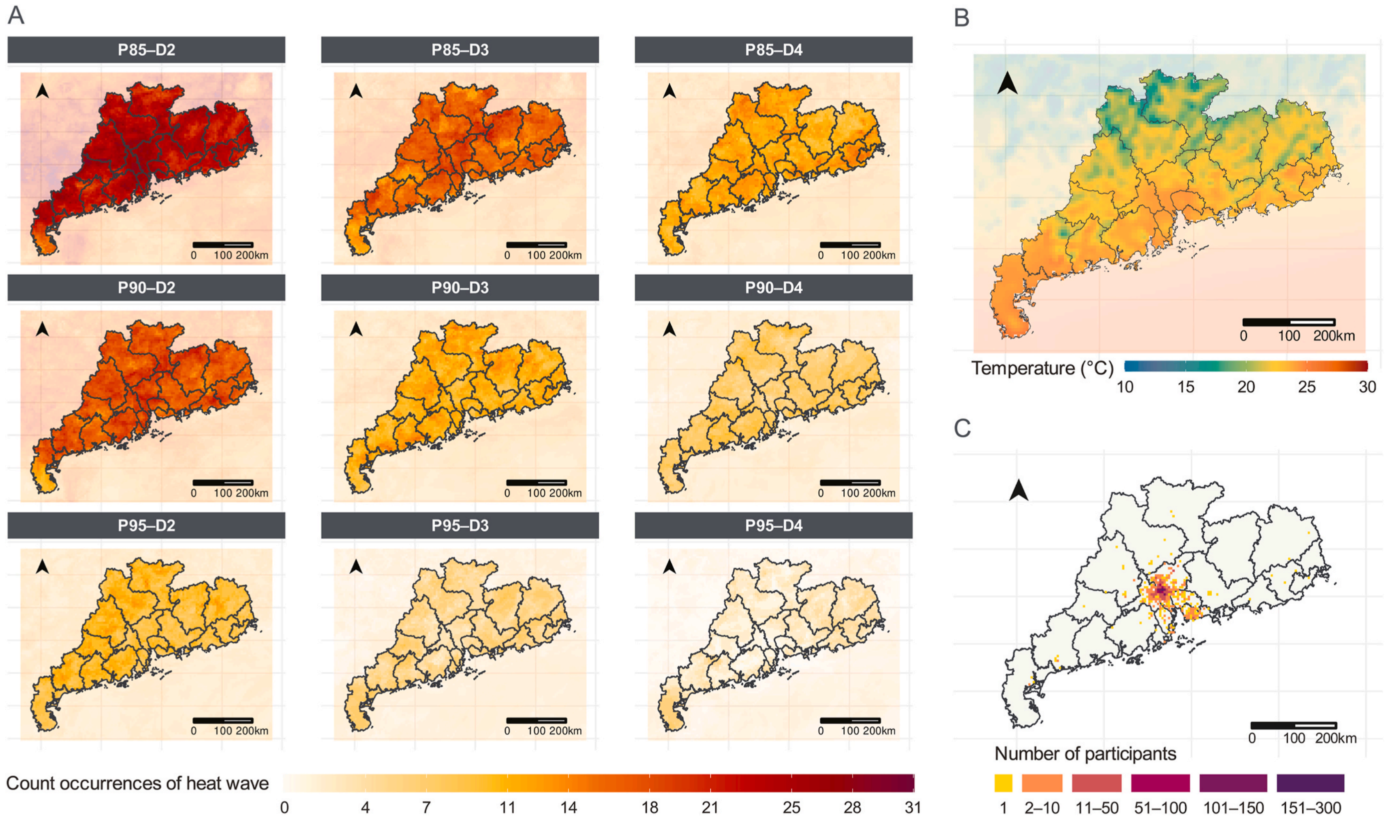

**Fig. 1.** Spatial distribution of the count occurrences of heat wave under different definitions (A), mean temperature (B), and the residential addresses of the participants (C) in Guangdong Province, China during 2018–2019. Detailed information for each heat wave definition is provided in Supplementary Table S1.

**Table 2**
Distribution of semen parameters and exposure to ambient temperature, relative humidity, and $PM_{2.5}$.

| Variable | Mean ± SD | P5 | P25 | P50 | P75 | P95 |
|---|---|---|---|---|---|---|
| Semen parameter | | | | | | |
| Semen volume, ml | 3.8 ± 1.5 | 1.7 | 2.8 | 3.6 | 4.6 | 6.5 |
| Sperm concentration, × $10^6$/ml | 76.4 ± 32.3 | 28.0 | 53.8 | 74.0 | 97.0 | 134.0 |
| Total sperm number, × $10^6$ | 277.9 ± 139.8 | 78.3 | 182.0 | 260.1 | 356.6 | 531.0 |
| Total motility, % | 59.6 ± 10.9 | 40.0 | 53.0 | 61.0 | 66.0 | 76.0 |
| Progressive motility, % | 57 ± 10.9 | 37.0 | 51.0 | 58.0 | 64.0 | 74.0 |
| Normal forms, % | 13.9 ± 6.5 | 4.9 | 9.0 | 13.0 | 17.8 | 25.4 |
| Meteorological variable and $PM_{2.5}$[a] | | | | | | |
| Mean temperature (°C) | 24.9 ± 4.0 | 17.5 | 21.8 | 26.0 | 28.5 | 29.6 |
| Mean relative humidity (%) | 79.9 ± 6.8 | 63.1 | 76.4 | 80.4 | 85.5 | 87.3 |
| Mean $PM_{2.5}$ concentration (μg/$m^3$) | 28.2 ± 6.8 | 18.3 | 22.3 | 27.9 | 34.1 | 38.7 |

SD = standard deviation; $PM_{2.5}$ = fine particulate matter.

[a] Mean temperature, mean relative humidity, and mean $PM_{2.5}$ concentration refer to the 90-day average values of the daily 24-h average temperature, relative humidity and $PM_{2.5}$ concentration during each of the 90 days before the date of each semen collection.

intraclass correlation coefficients (ICC) for each semen parameter, except for normal forms, were reported in Supplementary Table S5 (Keel, 2006).

### 3.2. Association of heat wave exposure with semen quality

As shown in Fig. 2, the association of heat wave exposure with semen quality varied across heat wave definitions. With the heat wave definition of P95−D4, compared to unexposed, exposed to heat waves within the 90-day period was significantly associated with a 0.11 (95% CI: 0.03, 0.18) ml, 3.36 (95% CI: 1.35, 5.38) × $10^6$/ml, 16.93 (95% CI: 7.95, 25.91) × $10^6$, and 2.11% (95% CI: 1.4%, 2.83%) reduction in semen volume, sperm concentration, total sperm number, and normal forms, respectively. With other heat wave definitions, these associations turned weaker or became insignificant. For total motility and progressive motility, exposed to heat waves under the definition of P90−D4 within the 90-day period was significantly associated with a 1.98% (95% CI: 1.47%, 2.48%) and 2.08% (95% CI: 1.57%, 2.58%) reduction, respectively. The numerical values corresponding to the results depicted in Fig. 2 are provided in Supplementary Table S3.

The sensitivity analysis conducted after excluding participants residing outside Guangzhou yielded results consistent with the primary results from the entire sample under the definition of P90−D4 and P95−D4 (Supplementary Table S4). Consistent results with the primary results were also obtained when including participants with illegible abstinence periods (Supplementary Fig. S3) or adjusting for age as a continuous variable (Supplementary Fig. S4). Under the P95–D3 and P95–D4 definitions, the estimates of total motility and progressive motility were greater after excluding participants aged over 30 (Supplementary Fig. S5). The number of heat waves experienced by individuals under different definitions was categorized, with the exception of P95–D2, P95–D3, and P95–D4, as experiencing multiple heat waves under these definitions is uncommon. The estimates of semen parameters are generally greater under multiple heat wave exposure compared to single heat wave exposure, but this trend appears to be reversed for total motility and progressive motility under the P90–D4 definition (Supplementary Fig. S6).

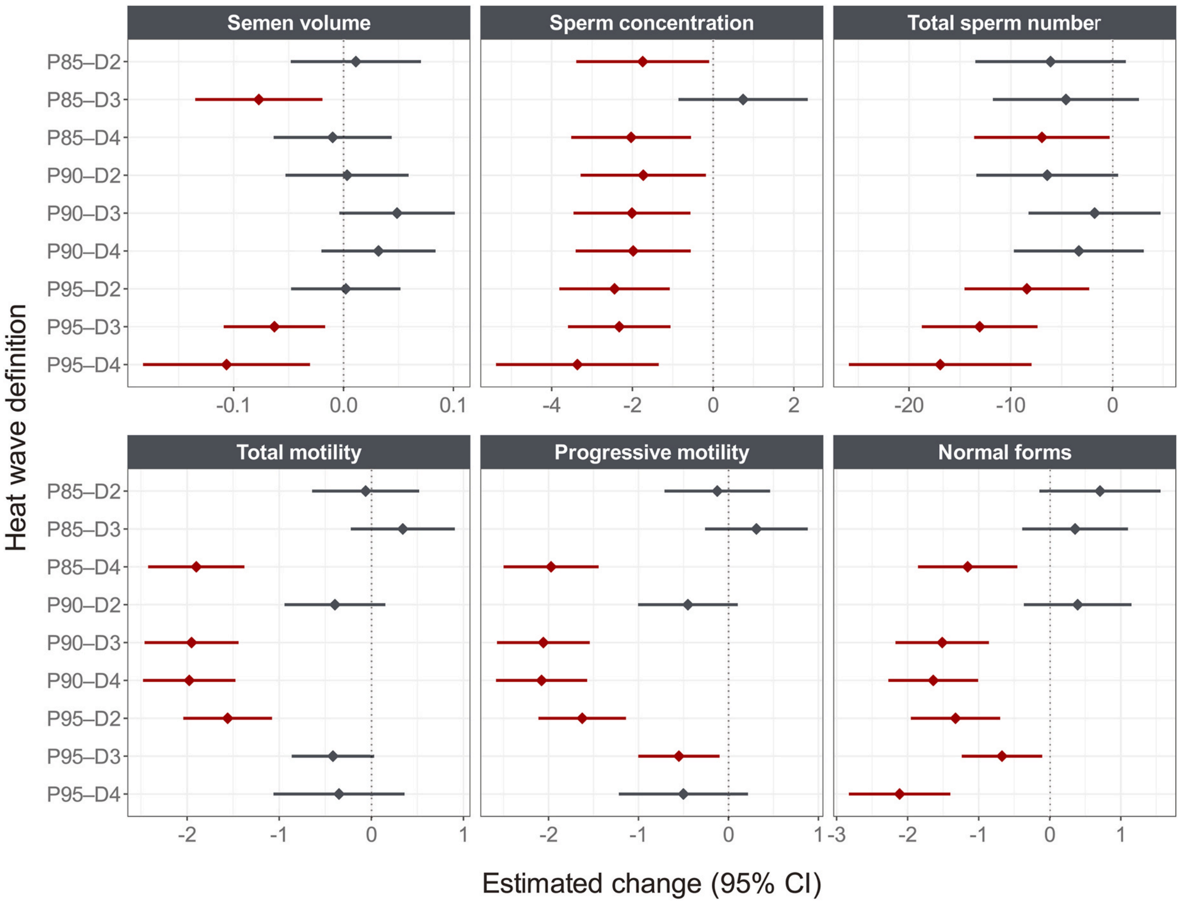

**Fig. 2.** Estimated change (95% CI) of semen parameters associated with exposure to heat wave under different definitions. Estimated changes with statistical significance are marked with red colors. All models were adjusted for age, educational attainment, marital status, fertility status, year at semen collection, season at semen collection, mean temperature, mean relative humidity, and mean $PM_{2.5}$ concentration. Detailed information for each heat wave definition is provided in Supplementary Table S1. CI = confidence interval; $PM_{2.5}$ = fine particulate matter.

### 3.3. Windows of susceptibility to heat waves

As shown in Fig. 3, under the total of 9 definitions from P85−D2 to P95−D4, the association of exposure to heat waves with a reduction in sperm concentration, total sperm number, and normal forms were observed in the first half (approximately 50 days), while the reduction in semen volume was observed in the middle third (approximately day 20–60) of the 90-day period. For total motility and progressive motility, exposure to heat waves under the P85−D2 to P90−D3 definitions in the last quarter (approximately day 70–90) of the 90-day period was significantly associated with a reduction in both parameters. The results of the empirical division were also consistent with the primary findings, as the direction of association for each of the four divided windows was in accordance with their corresponding period within the complete 90-day period (Supplementary Fig. S2).

## 4. Discussion

In this retrospective longitudinal study, we found that exposure to heat waves was significantly associated with a reduction in semen quality including sperm count, motility, and morphology. The identified windows of susceptibility varied across different semen parameters. Exposure to heat waves in approximately the first half of the 90-day period was significantly associated with a reduction in sperm count and morphology, while exposure in approximately the last 20 days of the 90-day period was significantly associated with the reduction of sperm motility.

This is the first study to quantitatively investigate the association between heat wave exposure and semen quality. Our findings are generally consistent with previous studies which suggest that exposure to non-optimum ambient temperatures, especially extreme high temperature, contributes to the decline of sperm count, motility and morphology (Thonneau et al., 1998; Wang et al., 2020; Zhang et al., 2013; Zhou et al., 2020). Taking into consideration that heat waves commonly occur in summer, our result is consistent with a previous study used a semi-quantitative approach and found semen volume, sperm concentration, motility, and the percentage of spermatozoa with normal morphology displayed trends opposite to average highest temperature variation, and were significantly lower in midsummer (Zhang et al., 2013). To date, limited studies quantified the association between ambient temperature and semen quality. An inverted U-shaped exposure-response relationship was identified by two studies based in Wuhan, China. One is our previous study among 10802 sperm donation volunteers, in which we identified an optimal exposure threshold of 13 °C (55.4 °F) during 0–90 days before semen collection for both sperm count and sperm motility (Zhou et al., 2020). The other study among 1780 men attending an infertility clinic divided the 90-day period into four exposure windows empirically, and thus provided further evidence that ambient temperature above the threshold in different exposure windows was associated with decrease of sperm concentration, progressive motility, and normal forms (Wang et al., 2020). It should be noted that Wuhan is an inland city with a subtropical monsoon climate in central China, featuring hot, humid summers and cold, dry winters. Guangdong however, is a subtropical coastal province that tends to have only two distinct seasons, among which the summers are longer and used to more extreme heat while in the winters the temperatures rarely

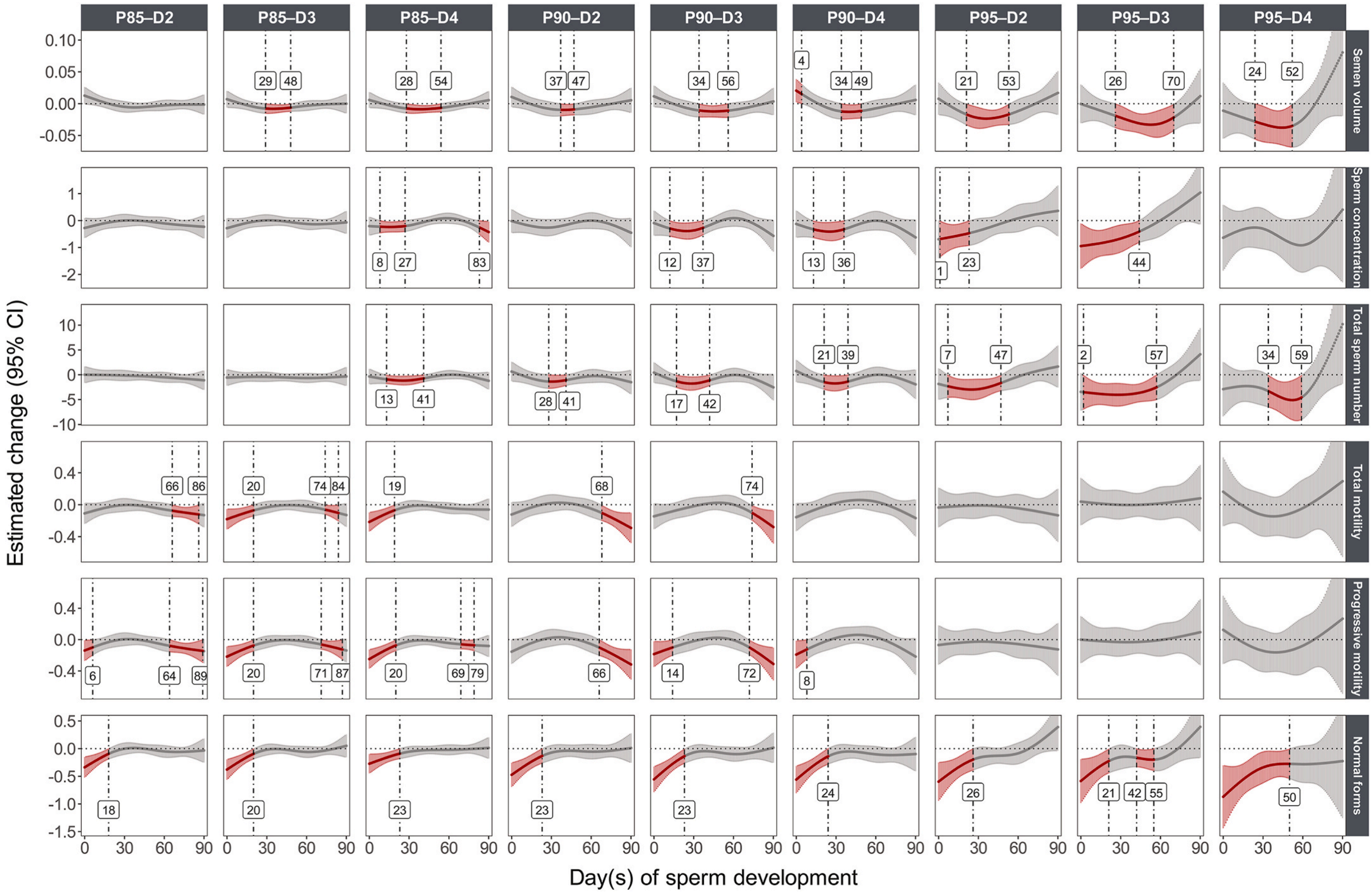

**Fig. 3.** Lag-response association of exposure to heat wave and semen quality. Days with significant effects are marked with red colors. Number labels represent the first or the last day with significant effects within the 90-day period of sperm development. All models were adjusted for age, educational attainment, marital status, fertility status, year at semen collection, season at semen collection, mean temperature, mean relative humidity, and mean $PM_{2.5}$ concentration. Detailed information for each heat wave definition is provided in Supplementary Table S1. CI = confidence interval; $PM_{2.5}$ = fine particulate matter.

drop below 13 °C (Ding et al., 2011). These climate features grant Guangdong an ideal region to study high ambient temperature and heat waves eliminating the confounding of cold. Our recent study conducted in Guangdong observed a negative association with a linear pattern but not an inverted U-shaped between ambient temperature and semen quality, indicating that ambient temperature in Guangdong is beyond a possible threshold of cold exposure to affect semen quality (Zhang et al., 2023). On the basis of these findings, the present study identified a significant association between heat wave exposure and the reduction in sperm count, motility, and morphology, and further corroborates the adverse effect of high ambient temperature on semen quality.

While other sensitivity analyses were consistent with the main result, under the P95–D3 and P95–D4 definitions, the estimates of total motility and progressive motility were greater after excluding participants aged over 30 (Supplementary Fig. S5). One possible explanation for the observed discrepancy in the age group could be differences in lifestyle preferences between younger and older participants. Conducting further studies with comprehensive data on the lifestyle preferences of individual participants could provide more evidence. Under the P90–D4 definition, which is the most extreme definition with the exception of P95–D2, P95–D3, and P95–D4, the trend of greater semen parameter estimates under multiple heat wave exposure compared to single heat wave exposure appears to be reversed for total motility and progressive motility, with the estimates under multiple heat wave exposure approaching statistically insignificant (Supplementary Fig. S6). Potential explanations for this observation could be that since the window of susceptibility is relatively narrower for sperm motility than sperm count and morphology, not all multiple exposures may necessarily occur during the window of susceptibility for sperm motility. Further, due to the limited number of participants who experienced multiple heat waves, the sample size for this analysis was relatively small. This observation deviates from initial expectations and warrants further investigation to better understand the underlying mechanisms or potential confounding factors.

Spermatogenesis is a complex and lengthy biological process of germ cell expansion and development through precisely timed and highly organized cycles, which has been simplified by germ cell association in time and thus divided into the 3 phases of spermatocytogenesis, meiosis, and spermiogenesis (Hess and De Franca, 2009; Sharma and Agarwal, 2011). The duration of each cycle in humans is reportedly 74 days, after adding in the epididymal transit time (estimated at 3–12 days) and abstinence interval, a 90-day period before semen ejaculation is generally considered sufficient to detect the impact of environmental factors exposure on any stage of sperm development (Selevan et al., 2000). Accordingly, 0–9 days before ejaculation corresponds to epididymis storage, 10–14 days before ejaculation corresponds to the development of sperm motility, and 70–90 days before ejaculation corresponds to spermatocytogenesis. With the application of DLNM, our findings suggest that heat wave exposure during an early stage (approximately 50 days) of spermatogenesis adversely affects sperm count and morphology, while the exposure during approximately 20 days before ejaculation leads to the decline of sperm motility, which were generally in line with the specific stages within the spermatogenesis period. This consistency suggests that either sperm count, morphology, or motility is more susceptible to heat wave exposure during their corresponding development stages. Moreover, compare to empirically divided exposure windows, the more accurate identification of potential windows of susceptibility on the time scale can enhance the insight into the potential mechanisms of the association between heat wave exposure and semen quality.

The potential biological mechanisms explaining the association between heat wave exposure and impaired semen quality in humans involve complicated pathways and crosstalk (Durairajanayagam et al., 2015). Heat stress can lead to apoptosis of germ cells by triggering oxidative stress, activating upstream signals such as p38 mitogen-activated protein kinase and caspase 2 (Hikim et al., 2003), and inducing DNA damage (Johnson et al., 2008; Luo et al., 2022; Shahat et al., 2020; Williams et al., 2019). Hormonal imbalances, including the down-regulation of the StAR gene and protein, often occur in response to heat stress, resulting in reduced blood concentrations of testosterone, impaired spermatogenesis, and compromised testicular integrity (Oka et al., 2017; Rizzoto et al., 2020). The aforementioned factors, along with the disruption of structural and metabolic support for germ cells provided by somatic testicular cells due to heat stress (Hagenas and Ritzen, 1976; Jegou et al., 1983; Vallés et al., 2014), contribute directly and indirectly to the loss of germ cells, ultimately leading to the decline in sperm number. Furthermore, heat-induced oxidative stress can also affect the structure and function of the sperm membrane through lipid peroxidation, which results in decreased sperm motility and increased percentages of morphological defects (De Lamirande and Gagnon, 1992; Hamilton et al., 2016).

It is interesting that the estimation of the association between motility parameters and exposure to heat waves did not increase with a more extreme definition, which differs from those of other semen parameters. This discrepancy could be explained by both the tendency of individuals to seek avoidance to exposure during more extreme heat wave days and the physiological characteristics of sperm, which exhibit high responsiveness to temperature fluctuations in their motility according to previous in vitro studies (Bahat et al., 2012; Boryshpolets et al., 2015). We hypothesize that individuals are more likely to change their behavior, such as using air conditioning and staying indoors, on more extreme heat wave days. Since sperm motility has higher responsiveness to temperature fluctuations than sperm count and morphology, this may ultimately contribute to the discrepancy in the association between exposure to extreme heat waves and different sperm parameters. However, the exact biological mechanism underlying the association between motility and heat wave exposure is not fully understood. It is crucial to interpret these results with caution, and further investigation, both in the laboratory and epidemiologically, is warranted to verify the findings of the current study before drawing any final verdict.

Our study has several advantages. First, the sample size of this longitudinal study was relatively large, and repeated semen analyses were helpful to better evaluate semen quality with consideration of intra-subject variations. Second, using a validated and high-resolution gridded dataset, we assessed exposure to heat waves based on each participant's residential address. The individual-level based exposure assessment can help reduce exposure misclassification, account for regional variations in climate and population acclimatization, and therefore provide more accurate estimates. Third, we conducted semen analysis strictly based on the WHO manual, which can provide us with reliable results of semen analyses and is important in comparing our results with future studies.

Nonetheless, some limitations exist. Despite the exposure assessment being individual-level based, the indoor temperature exposure was not assessed. Additionally, the data on individual activity trajectories (e.g., workplace location, outdoor activity preference) is unavailable in the current study. The lack of these data may result in an underestimation of heat wave exposure, as individuals often avoid outdoor activities and seek shelter in climate-controlled environments during the heat wave days, potentially biasing the estimation towards the null (Alberini et al., 2011; O'Lenick et al., 2020). Despite the potential underestimation of exposure to heat waves, a statistically significant association between exposure to heat waves and semen quality is still observed, suggesting that the association is likely to be robust. These findings could be confirmed and strengthened through further research with more detailed data on the individual activity trajectories of the participants, or continuous monitoring data of individual exposure through the use of wearable temperature detection devices. Lifestyle factors such as smoking and alcohol consumption may affect semen quality. However, we were unable to include them in our analysis due to a lack of data. Additional information such as the prevalence of these factors in the population being studied is required to determine the magnitude and direction of potential bias. Compared to general reproductive-aged men, the participants in the current study were sperm donation volunteers and generally younger and healthier, which may not sufficiently representative of the population. As a subtropical coastal province, Guangdong is accustomed to long, hot summers. It should be interpreted with caution while generalizing the results to regions with different climates.

## 5. Conclusion

In this longitudinal study, we found that exposure to heat waves during 0–90 days before ejaculation was significantly associated with a reduction in semen quality, and that the windows of susceptibility varied for sperm count, motility, and morphology. Our findings provide novel evidence for the adverse effects of heat wave exposure on semen quality, suggesting that heat wave exposure is a risk factor for the decline of semen quality. Reducing heat wave exposures (e.g., stay indoors during heat wave days and use an air conditioner) during 3 months before ejaculation can be helpful for those who intend to donate sperm or are expecting to conceive.

## Credit authors statement

**Xinyi Deng:** Software, Methodology, Formal analysis, Writing - Original Draft, Visualization, **Qiling Wang:** Investigation, Resources, Writing - Original Draft, **Chunxiang Shi:** Resources, Data Curation, Writing - Review & Editing, **Jing Wei:** Resources, Data Curation, Writing - Review & Editing, **Ziquan Lv:** Writing - Review & Editing, **Suli Huang:** Writing - Review & Editing, **Yong-Gang Duan:** Writing - Review & Editing, **Xinzong Zhang:** Conceptualization, Data Curation, Writing - Review & Editing, Supervision, Project administration, Funding acquisition, **Yuewei Liu:** Conceptualization, Methodology, Data Curation, Writing - Review & Editing, Supervision, Project administration, Funding acquisition.

## Funding

This work was supported by the Guangzhou Municipal Science and Technology Bureau [grant number 202201011581], the Guangdong Provincial Reproductive Science Institute [grant number C-01], and NHC Key Laboratory of Male Reproduction and Genetics, Guangdong Provincial Reproductive Science Institute, Guangdong Provincial Fertility Hospital [grant number KF201905]. The funders had no role in study design; data collection, analysis, and interpretation; writing of the article; and the decision to submit it for publication.

## Consent and ethics

The study was approved by the Ethics Committee of the School of Public Health, Sun Yat-sen University with a waiver of informed consent.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Data availability

The air pollution data are available at https://doi.org/10.5281/zenodo.6398971. The meteorological and clinical data are not publicly available.

## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envres.2023.116665.

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