

Supplemental File: Unpacking the Delhi Paradox: Seasonal Drivers of Sperm DNA Fragmentation

Supplemental Table 1: Participant demographic and clinical characteristics (n = 217)

Variable	Mean	Standard Deviation	Median	Range
Age (years)	36.01	4.93	35.00	22–50
BMI (kg/m ²)	26.98	4.23	26.40	14.40–41.90

Characteristic		Frequency (n)	Percentage (%)
Smoking	Yes	72	33.2
	No	145	66.8
Alcohol consumption	Yes	118	54.4
	No	99	45.6
Medication use	Yes	61	28.1
	No	156	71.9
Antihypertensive		9	4.1
Lipid-lowering		8	3.7
Diabetic		5	2.3
Thyroid		3	1.4
Other medication		4	1.8
Multivitamin use	Yes	36	16.6
	No	181	83.4
Medical comorbidity	Yes	34	15.7
	No	183	84.3
Hypercholesterolaemia		9	4.1
Hypertension		8	3.7
Diabetes mellitus		6	2.8
Hypothyroidism		3	1.4
Autoimmune disorder		3	1.4

Other comorbidity		9	4.1
Abstinence duration			
<1 day		46	21.2
1–3 days		142	65.4
>3 days		29	13.4

BMI = body mass index.

Supplemental Table 2: Seasonal meteorological parameters over the 10-year study period

Season	Months	Temperature (°C) Mean (SD)	Relative Humidity (%) Mean (SD)	AQI Mean (SD)
Winter	December– February	15.17 (2.21)	79.41 (5.76)	297.11 (53.40)
Pre- monsoon	March–May	27.92 (3.46)	54.10 (9.17)	191.98 (35.10)
Monsoon	June– August	31.40 (1.59)	71.42 (11.13)	109.28 (48.66)
Post- monsoon	September– November	26.58 (4.07)	74.82 (5.41)	176.30 (107.39)

AQI = Air Quality Index; SD = standard deviation. A notable decline in AQI was observed in 2020 due to the COVID-19 pandemic-related lockdown.

Supplemental Table 3: Association between air quality index (AQI) and semen parameters and DNA fragmentation index

Parameter	AQI <100 (n = 36)	AQI 101– 200 (n = 70)	AQI >201 (n = 111)	P value

Semen volume (mL)	2.30±1.07	2.07±1.14	2.03±1.04	0.325
Sperm concentration (×10 ⁶ /mL)	35.88±25.25	30.24±16.82	29.05±17.63	0.168
Progressive motility (%)	42.25±24.02	48.73±24.09	48.88±26.21	0.361
Immotile sperms (%)	27.72±24.77	17.70±18.93	19.09±21.65	0.056
Normal morphology (%)	15.64±10.69	14.11±10.87	17.28±13.43	0.474
TMSC (×10 ⁶)	25.71±21.39	26.94±25.62	23.52±23.43	0.600
DFI (%), median (IQR)	39 (26-61)	26.75 (20.62-36.38)	28.5 (20.5-38.5)	0.005*
DFI				<0.001*
≤15% (Excellent)	3 (8.3%)	7 (10.0%)	11 (9.9%)	
15.1–25% (Good)	6 (16.7%)	19 (27.1%)	31 (27.9%)	
25.1–50% (Poor)	15 (41.7%)	42 (60.0%)	64 (57.7%)	
>50% (Very Poor)	12 (33.3%)	2 (2.9%)	5 (4.5%)	

Values expressed as mean ± standard deviation unless stated otherwise. DFI is reported as median (interquartile range), consistent with the non-parametric statistical approach used for this variable; mean ± SD for DFI by AQI category was 44.82±25.18% (<100), 29.26±12.41% (101-200), and 29.65±11.71% (>201). *Statistically significant ($P < 0.05$). AQI = Air Quality Index; DFI = DNA Fragmentation Index; TMSC = Total Motile Sperm Count.

Supplemental Table 4: Association between ambient temperature and semen parameters and DNA fragmentation index

Parameter / Category	Mean Temperature °C (SD)	P value
Semen volume (mL)		0.545
0.5–2.4	24.21±6.96	
2.5–4.4	25.11±7.44	
4.5–5.5	25.21±6.68	

Liquefaction time			0.513
10–20 min	24.55±7.06		
21–30 min	24.32±7.22		
≥31 min	31.00±0		
Sperm concentration	Correlation (rho=-0.02)	Coefficient	0.785
Sperm concentration			0.187
<16×10 ⁶ /mL	25.45±7.25		
≥16×10 ⁶ /mL	24.22±7.00		
Progressive motility	Correlation (rho=-0.14)	Coefficient	0.034
Progressive motility			0.014*
<30%	26.33±6.75		
≥30%	23.95±7.06		
Normal morphology	Correlation (rho=-0.18)	Coefficient	0.008*
Sperm morphology			
<4%	25.76±6.76		0.138
≥4%	24.32±7.12		
TMSC			0.475
≤20×10 ⁶	24.94±7.11		
>20×10 ⁶	24.19±7.05		
DFI	Correlation (rho=0.09)	Coefficient	0.176
≤15% (Excellent)	24.19±7.39		0.064
15.1–25% (Good)	24.43±6.90		
25.1–50% (Poor)	23.95±7.29		
>50% (Very Poor)	28.95±4.06		

Values expressed as mean ± standard deviation unless stated otherwise. *Statistically significant ($P < 0.05$). rho = Spearman's correlation coefficient. DFI = DNA Fragmentation Index; TMSC = Total Motile Sperm Count.

Supplemental Table 5: Association between relative humidity and semen parameters and DFI

Parameter / Category	Mean Humidity % (SD)	P value
Semen volume		0.984 ¹
0.5 to 2.4 mL	70.41 ± 12.57	
2.5 to 4.4 mL	70.76 ± 12.62	
4.5 to 5.5 mL	71.14 ± 12.33	
Sperm concentration	Correlation Coefficient (rho=0.01)	0.846
Sperm concentration		0.387
<16×10 ⁶ /mL	69.38±13.10	
≥16×10 ⁶ /mL	70.98±12.31	
Progressive motility	Correlation Coefficient (rho=0.09)	0.204
Progressive motility		0.029*
<30%	67.60±13.21	
≥30%	71.56±12.10	
Normal morphology	Correlation Coefficient (rho=0.09)	0.173
Sperm morphology		0.121
<4%	68.18±12.68	
≥4%	70.99±12.47	
TMSC	Correlation Coefficient (rho=0.1)	0.137
		0.363
≤20×10 ⁶	69.58±13.23	
>20×10 ⁶	71.40±11.85	
DFI	Correlation Coefficient (rho=0.07)	0.298
≤15% (Excellent)	72.24±12.28	0.304
15.1–25% (Good)	67.91±13.36	

25.1–50% (Poor)	71.50±12.40		
>50% (Very Poor)	70.58±10.46		

Values expressed as mean $\pm$ standard deviation unless stated otherwise. *Statistically significant ($P < 0.05$). ρ = Spearman's correlation coefficient. DFI = DNA Fragmentation Index; TMSC = Total Motile Sperm Count.

Supplemental Table 6: Influence of demographic factors and semen parameters on sperm DNA fragmentation index (DFI)

Variable / Category	Median DFI (IQR)	Mean DFI (SD)	P value	X ² value
Season			0.009*	11.478
Winter	27.75 (20.62-37.62)	28.67±10.43		
Pre-monsoon	25.5 (19.5-31)	27.59±11.83		
Post-monsoon	31 (23.5-42.5)	35.02±18.92		
Monsoon	32.25 (24.5-45.5)	38.06±19.87		
Age group (years)			0.524	2.240
≤35	27 (20.5-39.5)	30.64±14.70		
36–40	29.5 (21.75-41.38)	33.48±18.60		
41–44	30.25 (25.5-40.88)	32.79±13.68		
≥45	31.5 (26.5-47)	35.55±15.44		
Smoking			0.132	
Yes		29.36±12.62		
No		33.37±17.26		
Alcohol consumption			0.108	
Yes		30.21±14.32		
No		34.23±17.52		

Abstinence duration			0.085	4.923
<1 day	25 (18.62-36.12)	27.07±11.13		
1–3 days	29.75 (22.5-41)	33.06±16.31		
>3 days	27.5 (22-49)	34.93±19.21		
Sperm concentration			<0.001*	19.557
<5×10 ⁶	42.5 (38.5-53.5)	48.50±21.88		
5–10×10 ⁶	38.5 (26.5-44.5)	36.13±12.47		
10–15.9×10 ⁶	29.75 (24.5-33.5)	28.75±9.93		
≥16×10 ⁶	26.5 (19.5-36.5)	30.23±15.20		
Sperm concentration			0.001*	W=5867.500
<16×10 ⁶ /mL	37.25 (26.5-42.5)	37.25±17.03		
≥16×10 ⁶ /mL	26.5 (19.5-36.5)	30.23±15.20		
Progressive motility			<0.001*	W=6916.000
<30%	41.25 (29.75-48.88)	43.61±20.38		
≥30%	26.5 (19.5-34.25)	27.83±11.48		
Normal morphology			<0.001*	W=4407.000
<4%	41.5 (29.5-49)	43.15±19.45		
≥4%	27.25 (20.5-36.5)	30.05±14.42		
TMSC			0.017*	W=6954.000
≤20×10 ⁶	31.25 (23.25-42.5)	34.62±17.18		
>20×10 ⁶	26.5 (20.5-36)	29.84±14.54		

DFI is reported as median (interquartile range), consistent with the non-parametric statistical approach used for this variable; mean ± standard deviation is retained as a secondary

reference. *Statistically significant ($P < 0.05$). DFI = DNA Fragmentation Index; TMSC = Total Motile Sperm Count.

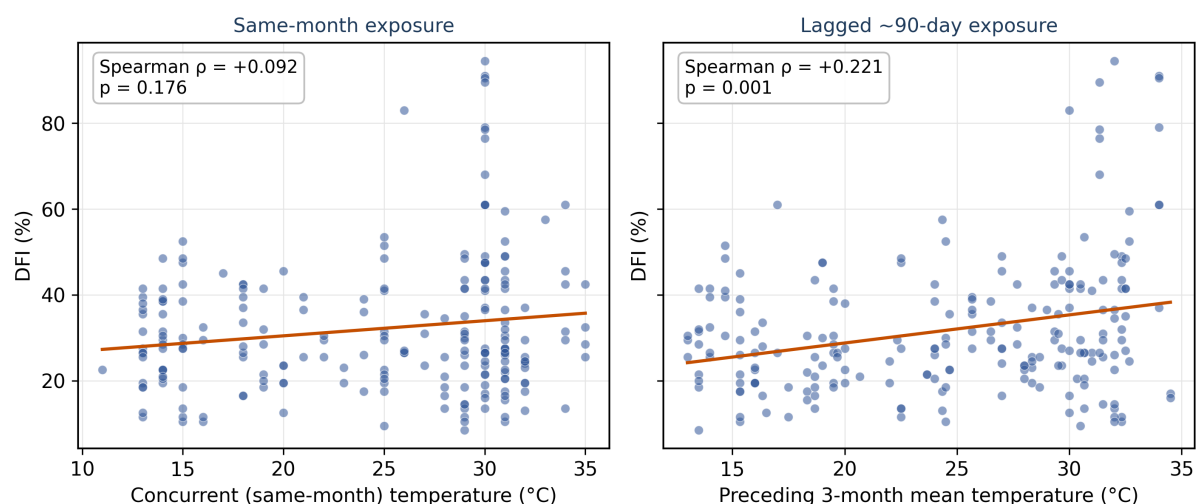
Supplemental Table 7: Contemporaneous versus lag-adjusted (~3-month) environmental correlations with sperm DNA fragmentation index (DFI)

Environmental variable	Contemporaneous rho	Contemporaneous P value	Lag-adjusted (~3-mo) rho	Lag-adjusted P value
Temperature	0.09	0.176	0.22	0.001*
AQI	-0.15	0.024*	-0.24	0.0005*
Humidity	0.07	0.311	-0.09	0.198

rho = Spearman's rank correlation coefficient. *Statistically significant ($P < 0.05$). Lag-adjusted values represent mean exposure over the ~90 days (three months) preceding sample collection of the 10-year study period. AQI = Air Quality Index; DFI = DNA Fragmentation Index.

DFI: same-month versus preceding-quarter temperature

DFI versus ambient temperature: same-month vs preceding-quarter window



Supplemental Figure 1: DFI against ambient temperature in the same month (left) and averaged over the three preceding months (right). Aligning exposure to the spermatogenesis window strengthens the positive association