

Original article

Association between underpants fit and semen quality: A hospital-based cross-sectional study

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Abstract

Background: The effect of the underpants type on semen parameters remains controversial. Therefore, conducting such a study in Thai male partners may provide valuable region-specific data.

Objectives: This cross-sectional study aimed to examine the association between underpants fit and semen parameters, including semen volume, sperm count, motility, morphology, and DNA fragmentation, among Thai male partners in Bangkok, Thailand.

Methods: Thai male partners aged 20–55 years who sought infertility treatment from the Reproductive Biology Unit, King Chulalongkorn Memorial Hospital, Bangkok, were recruited from March to June 2024. All participants completed a questionnaire on their sociodemographic, medical history, and underpants type and provided one semen sample, which was analyzed in accordance with the 6th edition of the World Health Organization laboratory manual (2021).

Results: Among the 116 eligible participants, 93.1% preferred wearing tight-fitting underpants during the day, whereas 74.1% opted for boxers or none at night. An increased semen volume was observed in men wearing loose-fitting underpants during the day and at night by 1.2 mL with $P = 0.004$ (95% CI: 0.41–2.06), whereas higher normal sperm morphology was found in men wearing tight-fitting underpants during that day and night by 0.5% with $P = 0.031$ (95% CI: 0.04–0.94). The other semen parameters were not significantly affected by the fit of the underpants.

Conclusion: A straightforward lifestyle choice, such as selecting a specific type of underpants, may influence semen volume and sperm morphology.

Keywords: DNA fragmentation, lifestyle factors, semen analysis, underpants fit.

Infertility is defined as the inability to achieve a clinical pregnancy after 12 months of regular, unprotected sexual intercourse or due to reproductive impairment in an individual or couple.⁽¹⁾ Global estimates from 1990 to 2021 suggest that approximately one in six people face infertility, with a lifetime prevalence rate of 17.5% (referring to the proportion of individuals encountering infertility at some point in their lives) and a period prevalence of 12.6% (referring to the proportion of individuals affected by infertility during a specific period, such as a given year).⁽²⁾ Furthermore, the prevalence of male infertility in the Southeast Asia region has increased by 121.6% from 1990 to 2021.⁽³⁾

In 20.0–30.0% of cases, men are the sole contributors, while they play a role in 50.0% of all infertility cases.⁽⁴⁾ Male infertility can arise from multiple factors, including health conditions, genetic abnormalities, infections, issues with the testes or surgeries impacting blood flow, and lifestyle habits.⁽⁵⁾ Lifestyle factors that can be modified are smoking⁽⁶⁾, alcohol intake^(7, 8), caffeine intake⁽⁹⁾, marijuana use⁽¹⁰⁾, obesity^(11, 12), heat exposure⁽¹³⁾, stress levels⁽¹⁴⁾, and choice of clothing.

Some research indicates that semen parameters, such as sperm motility, concentration, total sperm count, and DNA damage, vary depending on the type of underpants worn.^(15–17) Research indicates that the type of clothing worn, particularly pants and underpants, can increase the scrotal temperature by 1.5–2.0°C.^(18, 19) This increase subsequently raises the intratesticular temperature, contributing to the increased production of reactive oxygen species (ROS), which have been associated with reduced sperm motility and increased DNA damage.^(20, 21)

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Received: January 10, 2026

Revised: March 20, 2026

Accepted: April 28, 2026

The lack of enzymatic antioxidants, such as catalase, superoxide dismutase, and glutathione peroxidase, combined with deficiencies in non-enzymatic scavengers, including polyamines, taurine, lipoic acid, and vitamins C and E in sperm cells, may result in abnormal semen parameters.⁽²²⁾

Research on various lifestyle choices has provided inconsistent results. Similarly, the impact of underpants worn on semen parameters remains unclear, with previous and current studies yielding heterogeneous findings. This study aimed to explore the potential differences in semen parameters and sperm DNA integrity between individuals wearing loose-fitting vs. tight-fitting underpants.

Materials and methods

The study protocol was reviewed and approved by the Institutional Review Board of the Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand (Certificate of approval no. 1450/2023, date: October 31, 2023) and was conducted in accordance with the principles of the Declaration of Helsinki. In addition, this study was registered on ClinicalTrials.gov (Identifier: NCT06335706) on March 27, 2024, before participant recruitment began. Each participant provided written informed consent after receiving a full explanation of the study.

Study design

This cross-sectional study recruited Thai male partners seeking infertility treatment at the Reproductive Biology Unit at King Chulalongkorn Memorial Hospital in Bangkok, Thailand, between March and June 2024. The eligible participants were Thai males aged 20–55 years who volunteered to participate in this study. The exclusion criteria encompassed individuals with azoospermia, congenital or genetic disorders (such as Klinefelter syndrome, cystic fibrosis, cryptorchidism, and congenital absence of the vas deferens), recent genital tract conditions (including varicocele, testicular trauma, testicular torsion, erectile dysfunction, prostatitis, epididymitis, and related disorders), systemic diseases (such as diabetes, hypertension, dyslipidemia, or other similar conditions), cancer, current treatment regimens (including medications, chemotherapy, or radiation), and major surgeries affecting testicular vascularity (such as a vasectomy). Based on the equation reported by Samuel B. Green⁽²³⁾ ($N \geq 104 + m$), a sample size of 116 was estimated for a medium-sized partial correlation with 12 predictors, 0.80 power, and an alpha level of 0.05.

Questionnaire

Participants who agreed to participate in the study were asked to complete a 28-item self-reported questionnaire. This questionnaire covered socio-demographic information (including age and occupation), lifestyle factors (including type of underpants worn), habits (such as taking hot showers or using saunas), consumption of caffeine, alcohol, smoking, drugs, levels of stress, and exercise, as well as information on their medical history (recent infections, genitourinary tract disorders, surgical history, chronic diseases and their treatment, and previous fertility history).

The underpants were categorized as either loose-fitting (boxers) or tight-fitting (midway briefs, boxer briefs, trunks, briefs, bikini, jockstrap, and strings), with participants reporting usage separately during the day (≥ 8 hours/day) and at night.

Physical examination

Participants' weight (kg) and height (cm) were measured and documented on the form. Based on this information, the participant's body mass index (BMI in kg/m^2) was calculated.

Semen collection and analysis

All participants provided a semen sample via masturbation following the recommended 2–7 days of ejaculatory abstinence. The sample was collected in a sterile, wide-mouthed container, which was labeled with the participant's unique identification details, which included their hospital number and the date and time of collection. Abstinence duration was recorded upon sample receipt. Semen analysis was performed according to the 6th edition of the World Health Organization laboratory manual (2021).⁽²⁴⁾ The semen volume was recorded within the first 5 min and then incubated at 37°C. Macroscopic analysis included evaluating the semen liquefaction, appearance, viscosity, and pH. For all samples, microscopic analysis was performed using computer-aided sperm analysis, but for a few samples with oligospermia, a manual technique was employed. The analysis reported total sperm count (in millions), sperm concentration (in millions/mL), and various aspects of sperm motility (e.g., total motility, progressive motility, rapid-progressive motility, slow-progressive motility, non-progressive motility, and immotile sperm; all expressed as percentages), as well as sperm morphology (expressed as a percentage).

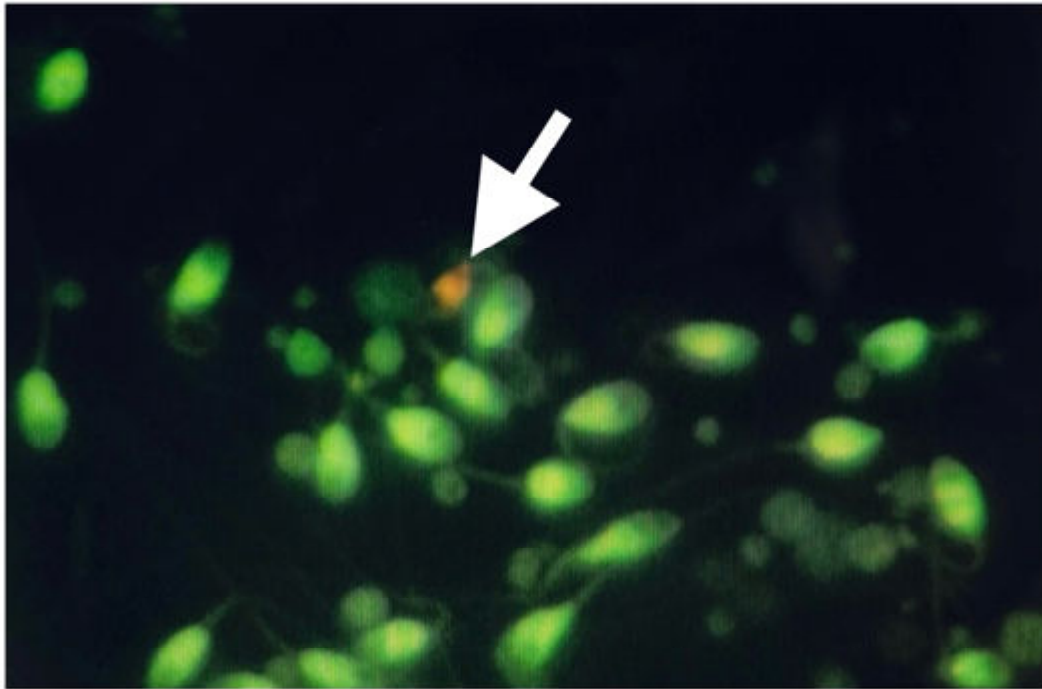


Figure 1. Fluorescence microscopy image of intact (green) and denatured (red, indicated by white arrow) sperm using the Acridine Orange test.

Sperm DNA integrity analysis

The acridine orange test was used to assess sperm DNA fragmentation. A 10 μ L aliquot of the semen sample was diluted with 1 $\times$ phosphate buffer solution, after which it was centrifuged, a smear prepared, and fixed overnight in Carnoy's solution to denature the chromatin, air dried, and stained with freshly prepared 1.0% (100 mg/10 mL) acridine orange solution for 5 min. It was then covered with a coverslip and examined using an Olympus BX50 fluorescence microscope with an oil-immersion objective. We counted 1,000 spermatozoa per slide. The sperm DNA fragmentation index (DFI) was calculated as the ratio of red (denatured) cells to the total number of red (denatured) and green (intact) cells. Immature sperm nuclei, characterized by high thiol-rich protamines, exhibit red fluorescence upon denaturation, whereas mature sperm nuclei, enriched with disulfide-rich protamines that resist DNA denaturation, emit green fluorescence (**Figure 1**).

Statistical analysis

The participants were categorized into three groups based on their underpants choices: Group 1 (LDN) wore loose-fitting underpants during the day and at night, Group 2 (TDLN) wore tight-fitting underpants during the day and loose-fitting underpants at night, and Group 3 (TDN) wore tight-fitting underpants during the day and at night. We assessed semen

parameters as continuous and categorical variables using the 6th edition of the World Health Organization laboratory manual (2021)⁽²⁴⁾ cut-offs and median values for the DFI. Demographic and categorical semen data were expressed as proportions, while continuous semen data were reported as means with 95% confidence intervals (CI) or medians with interquartile ranges depending on the distribution. We analyzed the categorical and continuous semen parameters using chi-square, ANOVA, and Kruskal–Wallis tests. In addition, we performed multiple linear and logistic regression models to adjust for confounding factors, including age, BMI, lifestyle factors, and abstinence duration. We conducted data analysis using STATA version 18, with statistical significance set at $P < 0.05$.

Results

The participant eligibility is outlined in detail in the flow diagram (**Figure 2**). A total of 161 individuals volunteered for the study, of whom 116 met the inclusion criteria. The three underpants-based groups were categorized according to lifestyle factors (**Table 1**), which revealed that most individuals in nearly all categories belong to the TDLN group. Analogously, semen parameters, as categorical and continuous variables, were summarized within the underpants-based groups.

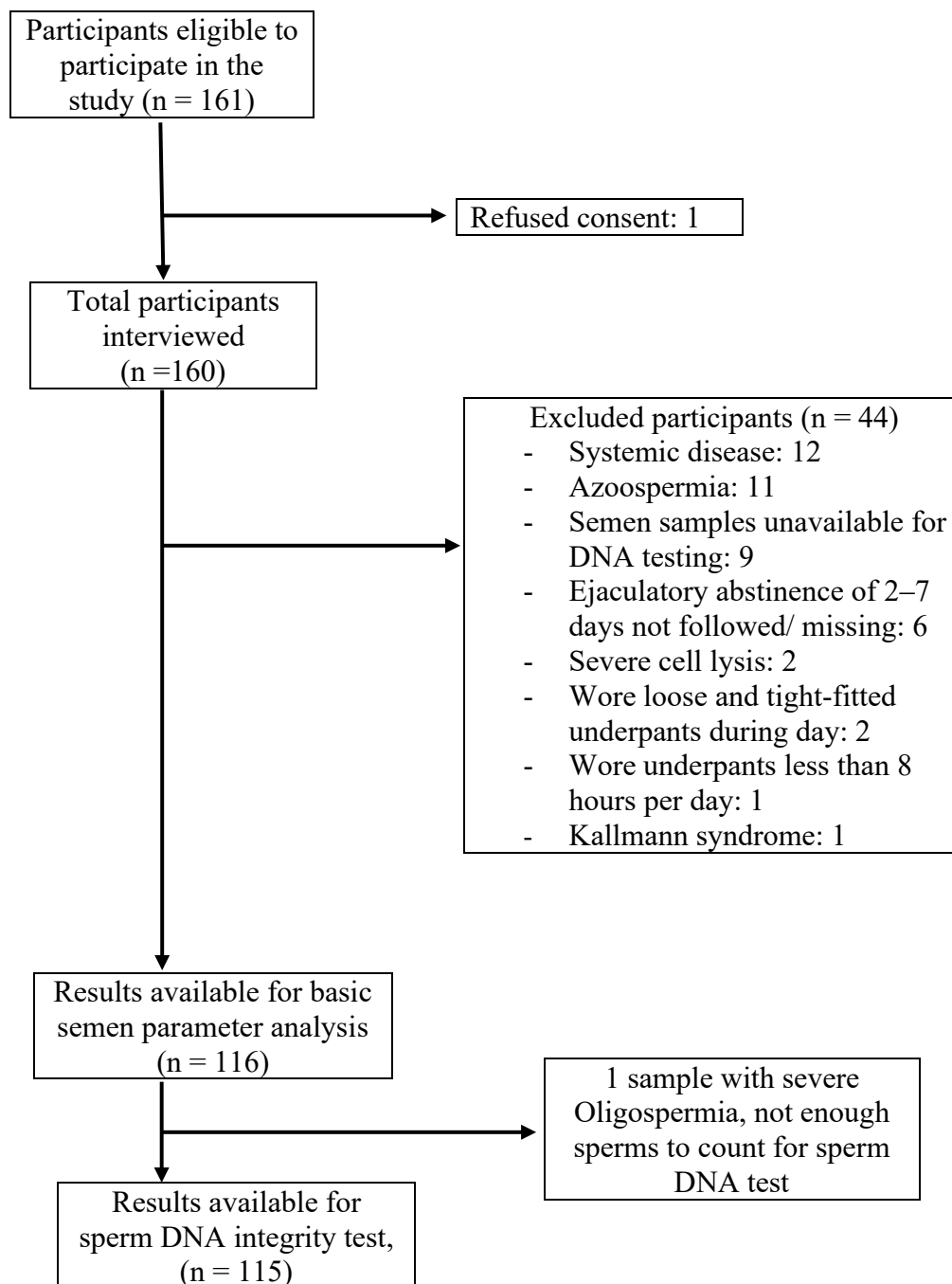


Figure 2. Flow diagram of the study participants.

As presented in **Table 2**, the analysis exhibited a higher semen volume, total sperm number, sperm concentration, and DFI when wearing loose-fitting underpants. In contrast, sperm motility and normal morphology were higher when wearing tight-fitting underpants. There was no significant difference in the semen parameters among the three groups ($P > 0.05$), whereas the Kruskal–Wallis test result revealed a significant difference ($P = 0.048$) (**Table 2**) in normal sperm morphology among the groups. Furthermore, when performing a pairwise test using Dunn's test, a significant difference was found between the TDLN and TDN groups ($P = 0.008$).

The unadjusted linear regression model revealed that the LDN group had a significantly higher semen volume by 1.2 mL ($P = 0.006$, 95% CI 0.35–2.04) compared to the TDLN reference group, with a β -coefficient of 2.8 (**Figure 3**). In addition, the TDN group showed a significantly higher normal morphology by 0.5% ($P = 0.031$, 95% CI 0.05–0.95) compared to the reference group, with a β -coefficient of 1.4 (**Figure 4**). These findings were corroborated by the adjusted model, which included age, stress, abstinence duration, and physical activity. In this adjusted analysis, the LDN group had a significantly increased semen volume of 1.2 mL ($P = 0.004$, 95% CI 0.41–2.06) with a β -coefficient of 3.2 (**Figure 3**),

Table 1. Demographic characteristics and lifestyle factors of the study participants.

Factors	LDN (n = 8); n (%)	TDLN (n = 78); n (%)	TDN (n = 30); n (%)
Age			
≤ 35 years (n = 42)	2 (4.8)	25 (59.5)	15 (35.7)
> 35 years (n = 74)	6 (8.1)	53 (71.6)	15 (20.3)
BMI (kg/m²)			
18.5 (underweight) (n = 1)	0 (0.0)	1 (100.0)	0 (0.0)
18.5-24.9 (normal) (n = 44)	6 (13.6)	28 (63.6)	10 (22.7)
25-29.9 (overweight) (n = 49)	1 (2.0)	37 (75.5)	11 (22.5)
> 30 (obese) (n = 22)	1 (4.6)	12 (54.6)	9 (40.9)
Physical activity			
No physical activity (n = 10)	0 (0.0)	6 (60.0)	4 (40.0)
Light moderate activity‡ (n = 41)	3 (7.3)	27 (65.9)	11 (26.8)
Moderate activity‡ (n = 28)	2 (7.1)	21 (75.0)	5 (17.6)
Moderate vigorous activity‡ (n = 35)	2 (5.7)	23 (65.7)	10 (28.6)
Strenuous activity‡ (n = 2)	1 (50.0)	1 (50.0)	0 (0.0)
Alcohol consumption (drinks per week)			
Do not drink (n = 37)	3 (8.1)	23 (62.2)	11 (29.7)
< 1 day/week (n = 63)	5 (7.9)	42 (66.7)	16 (25.4)
1–3 days/week (n = 12)	0 (0.0)	11 (91.7)	1 (8.3)
4–7 days/week (n = 4)	0 (0.0)	2 (50.0)	2 (50.0)
Caffeine consumption (glass per day)			
No (n = 6)	0 (0.0)	5 (83.3)	1 (16.7)
≤ 1/ day (n = 69)	7 (10.1)	43 (62.3)	19 (27.5)
2–3/ day (n = 41)	1 (2.4)	30 (73.2)	10 (24.4)
> 3/ day (n = 0)	0 (0.0)	0 (0.0)	0 (0.0)
Cigarette smoking			
Non-smoker (n = 78)	6 (7.7)	48 (61.5)	24 (30.8)
Former smoker (n = 16)	0 (0.0)	14 (87.5)	2 (12.5)
Current smoker (n = 22)	2 (9.1)	16 (72.7)	4 (18.2)
Recreation drugs use			
No (n = 115)	8 (7.0)	77 (67.0)	30 (26.1)
Yes (n = 1)	0 (0.0)	1 (100.0)	0 (0.0)
Stress (per last 3 months)			
No (n = 94)	6 (6.4)	65 (69.2)	23 (24.3)
Yes (n = 22)	2 (9.1)	13 (59.1)	7 (31.8)
Duration of abstinence			
2 days (n = 19)	0 (0.0)	12 (63.2)	7 (36.8)
3 days (n = 47)	3 (6.4)	34 (72.3)	10 (21.3)
4 days (n = 19)	1 (5.3)	13 (68.4)	5 (26.3)
5 days (n = 14)	3 (21.4)	9 (64.3)	2 (14.3)
6 days (n = 8)	1 (12.5)	2 (25.0)	5 (62.5)
7 days (n = 9)	0 (0.0)	8 (88.9)	1 (11.1)
Covid-19 infection (per last 3 months)			
No (n = 101)	8 (7.9)	66 (65.4)	27 (26.7)
Yes (n = 15)	0 (0.0)	12 (80.0)	3 (20.0)
Excessive heat exposure			
No (n = 58)	3 (5.2)	42 (72.4)	13 (22.4)
Yes (n = 58)	5 (8.6)	36 (62.1)	17 (29.3)

‡ Light moderate activity: daily household work; moderate activity: walking or jogging for at least 30 minutes daily; moderate-vigorous activity: muscle strengthening training 3 times a week for at least 20 minutes; strenuous activity: sports training several times a week. Non-smoker: never smoked or smoked fewer than 100 cigarettes in lifetime; former smoker: smoked at least 100 cigarettes in lifetime but had quit; current smoker: smoked 100 cigarettes in lifetime and continues smoking. LDN, loose-fitting underpants day and night; TDLN, tight-fitting underpants during day and loose-fitting at night; TDN, tight-fitting underpants day and night.

Table 2. Comparison of semen parameter values among recruited participants.

Semen parameter	LDN (n = 8)	TDLN (n = 78)	TDN (n = 30)	P-value
Immotile sperm [†] (%)	47.7 (37.7–57.7)	43.2 (38.4–47.9)	44.5 (36.7–52.4)	0.228
Non-progressive motility [†] (%)	7.4 (5.7–9.1)	7.1 (6.3–7.7)	6.3 (5.2–7.5)	0.449
Rapid progressive motility [†] (%)	34.8 (26.1–43.5)	40.0 (36.1–44.0)	40.3 (33.7–46.9)	0.271
Progressive motility [†] (%)	44.9 (35.7–54.0)	49.8 (45.5–54.2)	49.2 (42.0–56.3)	0.228
Total motility [†] (%)	52.3 (42.3–62.3)	56.8 (52.1–61.6)	55.5 (47.6–63.3)	0.228
DFI [§] (%)	66.1 (37.2–74.7)	52.1 (18.3–74.4)	57.0 (12.6–88.0)	0.655
Normal forms [§] (%)	1.1 (0.8–1.7)	1.2 (0.5–1.9)	1.8 (1.1–2.2)	0.048*
Sperm concentration [§] (10v/ml)	77.1 (32.5–102.5)	68.5 (35.2–115.1)	50.7 (36.2–82.1)	0.344
Total sperm number [§] (10v/ejaculate)	269.8 (171.0–359.5)	199.0 (92.9–326.6)	146.9 (85.9–224.7)	0.130
Semen volume [§] (ml)	2.9 (2.4–5.9)	2.8 (2.1–3.7)	2.7 (1.9–3.8)	0.416

Data are presented as mean (95% CI) for normally distributed variables and median (IQR) for non-normally distributed variables.[§] Non-normally distributed variables.[†] Normally distributed variables.* $P < 0.05$ indicates statistical significance (bold), P -values derived using one-way ANOVA for normally distributed variables and Kruskal-Wallis test for non-normally distributed variables. DFI, DNA fragmentation index; LDN, loose-fitting underpants day and night; TDLN, tight-fitting underpants during day and loose-fitting at night; TDN, tight-fitting underpants day and night.

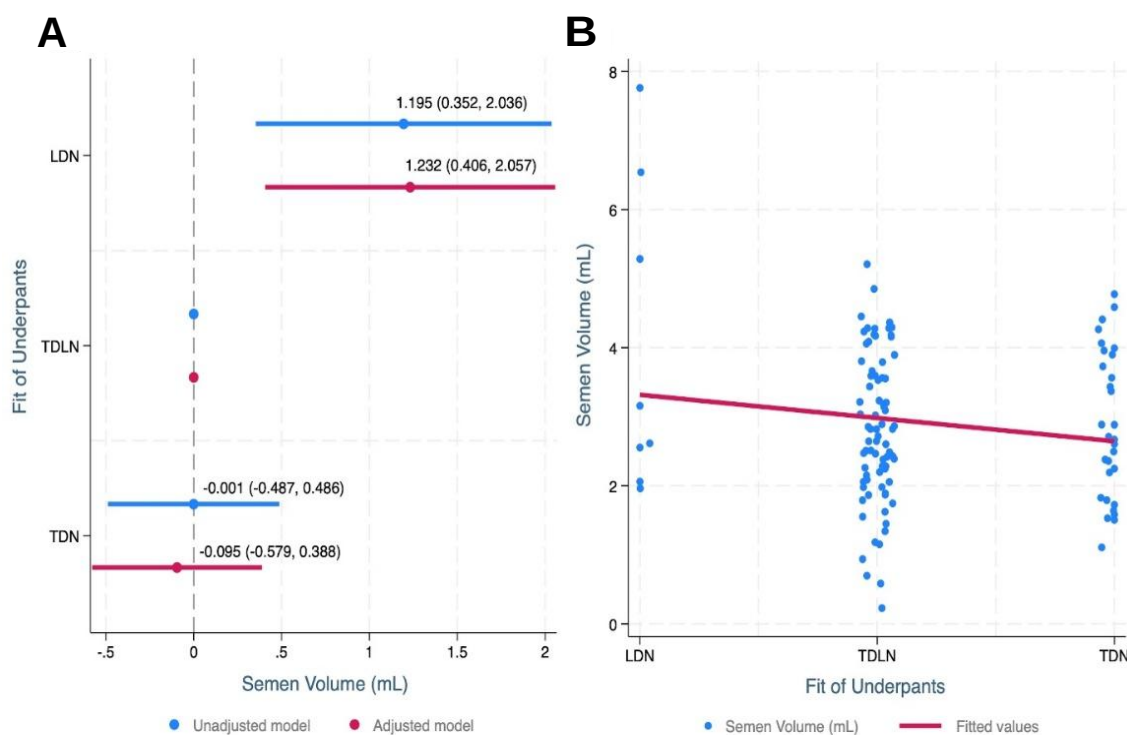


Figure 3. Coefficient and scatter plots illustrating the correlation between underpants fit and semen volume. (A) Coefficient plot of group on semen volume, (B) scatter plot of semen volume and group with fitted regression line. LDN, loose-fitting underpants day and night; TDLN, tight-fitting underpants during the day and loose-fitting at night; TDN, tight-fitting underpants day and night.

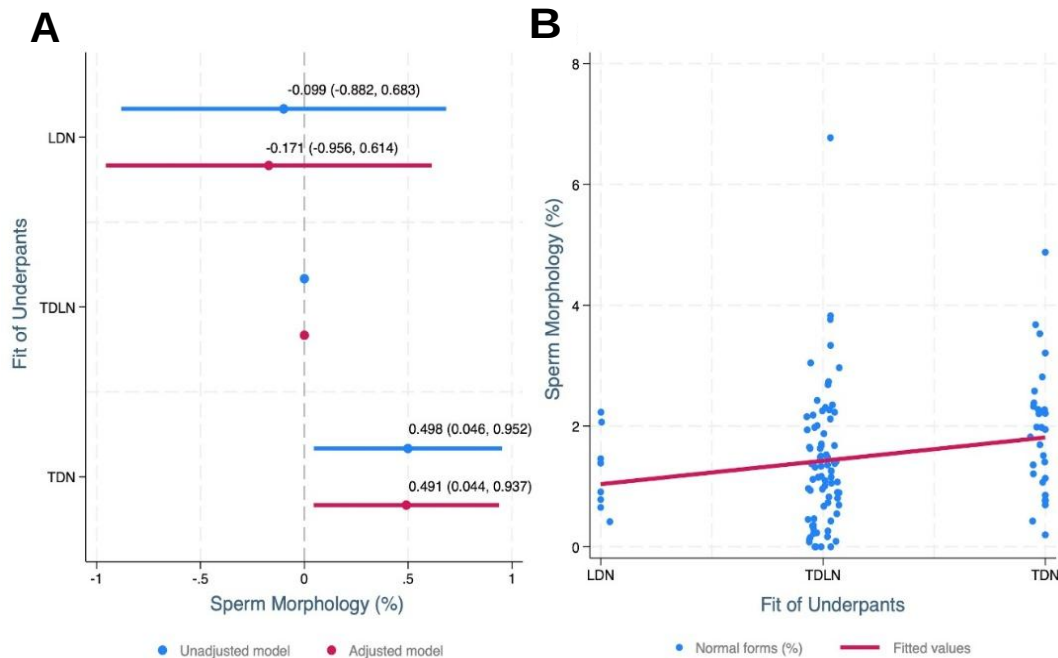


Figure 4. Coefficient and scatter plots illustrating the correlation between underpants fit and sperm morphology. (A) Coefficient plot of group on sperm normal morphology, (B) scatter plot of sperm normal morphology and group with fitted regression line. LDN, loose-fitting underpants day and night; TDLN, tight-fitting underpants during the day and loose-fitting at night; TDN, tight-fitting underpants day and night.

and the TDN group had a significantly higher normal morphology by 0.5% ($P = 0.031$, 95% CI 0.04–0.94) with a β -coefficient of 1.8 (Figure 4). No other semen parameters exhibited significant differences among the groups relative to the reference group in either model. Moreover, the logistic regression analysis revealed no significant differences in semen parameters between the groups in both models.

Discussion

This cross-sectional study revealed that the majority of Thai male participants preferred wearing TDLN. In accordance with our hypothesis, regression analysis indicated a significant positive relationship between wearing loose underpants during the day and at night and a higher semen volume. This finding aligns with previous studies, which reported that tight-fitting underwear increases testicular stress, thereby reducing semen volume.^(25–27) This result is further supported by the heat stress theory, which suggests that elevated intrascrotal temperature generates ROS that impair semen quality.^(20, 22, 28) Recent research has found a significantly higher prevalence of low semen volume (<1.5 mL) in infertile men (16.7%) compared to that of fertile men (4.0%).⁽²⁹⁾ In addition, another study identified a lower fertilization rate associated with low

semen volume. However, their study suggested that semen parameters were not predictive of assisted reproductive technology outcomes.⁽³⁰⁾

While the heat stress theory posits that wearing loose-fitting underpants should enhance all semen parameters, our findings reveal that sperm motility was better in men who wore tight-fitting underpants. This contrasts with a study that reported significantly decreased total motility and progressive motility in men wearing tight-fitting underpants.⁽¹⁸⁾ This implies that additional factors may be responsible for these variations in sperm motility results, including sperm acidic pH (5.2–6.2)⁽³¹⁾, mitochondrial DNA mutations⁽³²⁾, diet (organ meat)⁽³³⁾, and lack of sleep.⁽³²⁾ Furthermore, contrary to the findings of a few previous studies, which suggested no association between the type of underpants worn and sperm morphology^(34, 35), and an alternative study that reported an odds ratio of 1.9 for decreased normal morphology in men wearing tight-fitting underpants⁽²⁶⁾, we found a significant positive relationship between normal sperm morphology and wearing tight-fitting underpants during the day and at night. These differing results might be attributed to other factors that affect sperm morphology, such as genetic variation, polymorphisms, or functional impairments.⁽³⁶⁾

In this present study, the DFI was highest in men wearing loose-fitting underpants during the day and at night (66.1%) and lowest in the TDLN group (52.1%). However, regression analysis revealed no significant association between the type of underpants worn and DFI. This finding aligns with a 2018 cross-sectional study, which similarly found no significant association.⁽¹⁷⁾ Conversely, a 2014 cross-sectional study found a significant negative association between wearing boxer shorts and the DNA damage percentage in univariate and multivariate models.⁽²⁸⁾ In addition, a population-based preconception cohort study reported that men wearing boxers during the day and no underpants at night exhibited a decreased DFI.⁽³⁷⁾ Sperm DNA fragmentation can be influenced by several factors. While elevated scrotum temperatures may cause oxidative damage to sperm DNA, this type of damage does not always result in fragmentation. The fragmentation process is not solely dependent on oxidative stress, as it can also be driven by issues such as inadequate chromatin maturation in sperm cells and the process of programmed cell death, i.e., apoptosis.⁽²¹⁾

These conflicting results suggest that the type of underpants worn may affect intrascrotal heat but does not uniformly affect all semen parameters. Our results indicate that wearing loose-fitting underpants improves semen volume, total sperm count, and sperm concentration but does not enhance sperm motility and morphology. This suggests that the effect of underpants choice on semen quality is parameter-specific and does not operate in isolation. Instead, it interacts with other factors to influence the overall semen quality. Semen quality may also be influenced by the seminal micronutrient levels, including zinc, copper, selenium, manganese, and various vitamins, which is an area of ongoing research that warrants further investigation.⁽³⁸⁾

This study has several strengths. Data were collected through real-time interviews, thereby avoiding reliance on outdated medical records. Participants were interviewed before semen collection, thus reducing bias. Linear and logistic regression analyses were both employed to adjust for confounding factors, which ensured robust analysis. The Acridine Orange test is recognized for its simplicity and cost-effectiveness. In addition, a single scientist assessed the DNA fragmentation index to minimize variability.

However, some limitations should be noted. The cross-sectional design limits causal inference. Self-

reported underpants may introduce reporting bias, although intentional inaccuracies were unlikely. Potential confounders, including fabric type, elasticity, and consistency of use, were not assessed because of reporting constraints. Furthermore, selection bias may be present, as participants were recruited from a single center. In addition, unequal subgroup sizes and reliance on a single semen analysis may affect the reliability of the findings. Lastly, the Acridine Orange test is limited by fluorescence fading.

Conclusion

These findings suggest that a simple lifestyle choice, such as selecting a specific type of underpants, may influence certain semen parameters. However, the clinical significance of these differences remains unclear, as their effect on fertility outcomes cannot be determined. In addition, as this study was conducted among infertile men from a single center, the findings might not be generalizable to the broader population. Therefore, further large-scale studies in diverse populations are required to validate these results and clarify their clinical relevance.

Author contributions

HK, UJ, and PT designed the research study. UJ was responsible for project administration, supervision, and securing research funding. HK and JL collected the data, performed statistical analyses, and interpreted the results. PT and CS were responsible for gathering and organizing laboratory materials. HK drafted the original manuscript. PT, CS, and JL conducted a critical review and editing of the manuscript. All authors approved the final version submitted for publication and take responsibility for statements made in the published article.

Acknowledgements

This research project received support from the Ratchadaphisek Research Fund Type 1 at Chulalongkorn University (grant no. GA67/037). The authors sincerely thank Ms. Niramol Mankhong, Ms. Gade Porntawansak, Ms. Nantana Thongrod, and Ms. Rachanee Wongwathanavikrom for their assistance in interviewing the participants. They also appreciate the contributions of research scientists Ms. Jiraporn Ngeamvijiwat and Mr. Pattara Singchardcharn and their colleagues for semen sample analysis, as well as Dr. Yuda Chongpison for her support with data analysis.

Conflicts of interest statement

The authors have each completed the International Committee of Medical Journal Editors Form for Disclosure of Conflicts of Interest and declare that there are no conflicts of interest related to the conduct, authorship, or publication of this study.

Data sharing statement

The datasets generated and analyzed during this study are included in this published article. Further details are available from the corresponding author upon reasonable request for non-commercial purposes.

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