**ORIGINAL RESEARCH ARTICLE**

**The Role of X and Y Chromosomes in Semen Morphology and Concentration: A Study in Saran, Bihar, India**

**Abstract**

This study investigates the age-related variations in body mass index (BMI), ethnicity, abstinence time, smoking status, and sperm quality among men from Saran, Bihar, across six age groups (20–50 years). BMI demonstrated a progressive increase with age, ranging from 22.5 ± 1.5 kg/m² in the youngest group (20–25 years) to 28.5 ± 2.0 kg/m² in the oldest group (46–50 years), highlighting a trend of increasing weight with age. Ethnic distribution revealed a predominance of Hindus across all groups, with a decline from 75% in younger men to 50% in older individuals, accompanied by a proportional increase in Muslims. Abstinence time showed notable age-related variations, with shorter abstinence periods (≤2 days) more common in younger participants, and longer periods (≥5 days) more prevalent in older groups. Smoking behaviour exhibited a marked shift, with the percentage of never-smokers decreasing from 62.5% in the youngest group to 30% in the oldest, while the prevalence of current smokers increased correspondingly. Sperm quality analysis revealed significant age-related declines in sperm concentration (from 45 ± 5 million/mL to 15 ± 3 million/mL), normal morphology (from 75% to 50%), and motility (from 80% to 40%). Chromosome distribution analysis indicated a strong correlation between higher proportions of Y-chromosome-bearing sperm and better semen quality. Younger age groups, characterized by higher percentages of Y chromosomes (56% in Group A), exhibited superior sperm concentration, morphology, and motility, while older groups with reduced Y-chromosome proportions (49% in Groups E and F) showed deteriorated semen parameters. These findings underscore the combined impact of aging, lifestyle factors, and chromosomal distribution on male fertility. The study highlights the potential of Y-chromosome dominance as a biomarker for assessing semen quality, emphasizing the need for early health interventions to preserve reproductive health in men.

**Keywords**: Sperm, X chromosome, Y chromosome, semen morphology, semen concentration.

**Introduction**

The male gamete, the sperm cell, carries one of two possible sex chromosomes: either the X chromosome or the Y chromosome. These chromosomes play a crucial role in determining the sex of the offspring. The presence of an X chromosome in the sperm cell results in the birth of a female child (XX), whereas the Y chromosome leads to the birth of a male child (XY) (Gellatly,2009). While the primary function of these sex chromosomes is the determination of offspring sex, emerging studies suggest that the chromosomal composition of sperm may have broader implications for male fertility. Specifically, sperm cells carrying the Y chromosome have been observed to exhibit better semen quality—marked by higher sperm concentration and improved morphology—while sperm cells carrying the X chromosome are often linked to lower semen quality, including reduced concentration and structural abnormalities in sperm. Certain preliminary studies reported several morphological differences between the X and Y spermatozoa using phase-contrast microscopy (Shettles, 1960; Cui and Matthews, 1993; Cui, 1997); however, most of the recent studies indicate that no major differences exist between the two sperm types (Hossain et al., 2001; You et al., 2017) except their DNA content.

Semen quality is a critical determinant of male fertility, encompassing several key parameters such as sperm concentration, morphology, and motility. These characteristics are essential for successful fertilization. Abnormalities in any of these parameters, such as low sperm concentration or poor sperm morphology, can lead to infertility or subfertility. Beyond fertility, semen quality is also seen as an indicator of overall male health. Poor semen quality has been associated with a range of health issues, including genetic disorders, environmental toxicants, and lifestyle factors such as diet, smoking, and alcohol consumption. Furthermore, semen quality can be influenced by various external factors (Jurewicz,2009), including environmental pollution and exposure to agricultural chemicals, which may be particularly relevant in regions with distinct urban and rural populations.

The male population of Saran, Bihar, provides a unique and valuable context for investigating the relationship between sperm sex chromosomes and semen quality. Saran, located in eastern part of India, consists of both urban and rural populations with varied lifestyles, environmental exposures, and healthcare access. Rural areas are often more exposed to agricultural chemicals and traditional practices, while urban areas may have higher levels of industrial pollutants and modern lifestyle-related health risks. Understanding how these different factors interact with sperm chromosomal composition could provide critical insights into male reproductive health in this region.

**Aim of the Study**

This study aims to investigate the role of sperm sex chromosomes—X and Y—in determining semen morphology and concentration in the male population of Saran, Bihar. Sperm bearing the Y chromosome are more likely to be associated with higher sperm concentrations and better sperm morphology compared to those carrying the X chromosome. This study seeks to contribute to the growing body of knowledge on the genetic factors that influence semen quality and to explore the potential implications for male fertility in the context of environmental and lifestyle factors specific to Saran, Bihar.

**Materials and Methods**

**Study Design**
A study was conducted in Saran, Bihar, India, with 200 male participants representing both rural and urban populations. Participants were selected to capture a diverse sample of the male population, ranging in age from 20 to 50 years. The study cohort included both healthy individuals with normal semen quality and those diagnosed with suboptimal semen quality, characterized by low sperm concentration, abnormal morphology, or reduced motility. This diverse group was chosen to examine the potential influence of sperm chromosomal composition on semen quality across varying health statuses, environmental exposures, and lifestyle factors

**Semen Sample Collection and Analysis**
Semen samples were collected from participants after a 3–5-day period of sexual abstinence, which is recommended to standardize sperm concentration and motility (World Health Organization [WHO], 2021). The samples were provided in sterile containers and immediately transported to the laboratory for analysis. Semen analysis was performed in accordance with the guidelines set by the WHO for semen examination (WHO, 2021) and the following parameters were assessed:

**Semen Concentration**:
The sperm concentration was measured using a hemocytometer, a precision instrument for counting sperm cells in a defined volume. The results were expressed as sperm cells per milliliter (million/mL). A sperm concentration of less than 15 million sperm/mL was considered suboptimal based on WHO reference values (WHO, 2021).

**Sperm Morphology**:
Sperm morphology was evaluated under a light microscope following standard staining techniques to highlight structural abnormalities. Sperm cells were classified as "normal" or "abnormal" based on defects in the head (e.g., large, misshapen, or tapered heads), mid-piece (e.g., coiled or broken), or tail (e.g., bent, short, or multiple tails). The proportion of normal sperm was calculated as a percentage of the total sperm examined. Morphological abnormalities in sperm are associated with potential fertility issues (Winters and Walsh,2014).

**Sperm Motility**:

Sperm motility was evaluated by determining the percentage of sperm exhibiting progressive forward movement. Sperm were categorized as Grade A (fast, straight-line movement), Grade B (slow, straight-line movement), Grade C (slow, non-linear movement), and Grade D (immotile). A motility of less than 40% progressive motility was considered suboptimal according to WHO standards (WHO, 2021).

**Genetic Analysis**
To assess the chromosomal composition of sperm, Fluorescence in situ Hybridization (FISH) was employed. FISH is a highly sensitive technique used to detect specific chromosomes within individual sperm cells (Ryan Bishop,2010). In this method, specific fluorescent probes were used to bind to the X and Y chromosomes. Fluorescent signals were captured using a fluorescence microscope, allowing differentiation of sperm cells carrying either the X or Y chromosome.

**FISH Procedure**:
Sperm samples were fixed on slides and hybridized with DNA probes specific to the X and Y chromosomes. After hybridization, the slides were washed, and the signals were analysed under a fluorescence microscope. The number of X-bearing and Y-bearing sperm cells was recorded for each sample, and the proportion of each type of sperm was calculated. This procedure enabled accurate determination of the X-to-Y chromosome ratio in the semen samples.

**Statistical Analysis**

Descriptive statistics were performed to summarize the characteristics of the study population, including age, semen concentration, sperm morphology, and motility. The mean, median, and standard deviations were calculated for continuous variables, while proportions were used for categorical variables.

**Chi-Square Test**:
To assess the statistical significance of the relationship between sperm chromosomal composition (X and Y chromosomes) and semen quality parameters (concentration, morphology, and motility), the chi-square test was employed. This test allowed the comparison of proportions of X-bearing and Y-bearing sperm with respect to different semen quality parameters.

**Correlation Analysis**:
Pearson correlation coefficients were computed to determine the strength and direction of the relationship between sperm concentration, morphology, and the proportion of X and Y chromosome-bearing sperm. A positive correlation would indicate that an increase in the proportion of Y chromosome-bearing sperm is associated with better semen quality, while a negative correlation would suggest the opposite.

**Results**

Ta**ble-1; Age-Grouped Analysis of BMI, Ethnicity, Abstinence Time, and Smoking Status in the people of Saran, Bihar.**

| **Parameter** | **Group A (20–25 years)** | **Group B (26–30 years)** | **Group C (31–35 years)** | **Group D (36–40 years)** | **Group E (41–45 years)** | **Group F (46–50 years)** |
| --- | --- | --- | --- | --- | --- | --- |
| **Number of People** | 40 | 40 | 40 | 40 | 20 | 20 |
| **BMI (kg/m², mean ± SD)** | 22.5 ± 1.5 | 24.0 ± 2.0 | 25.0 ± 2.5 | 26.5 ± 2.0 | 27.0 ± 2.5 | 28.5 ± 2.0 |
| **Ethnicity [n (%)]** |  |  |  |  |  |  |
|   Hindu | 30 (75%) | 28 (70%) | 26 (65%) | 25 (62.5%) | 12 (60%) | 10 (50%) |
|   Muslim | 8 (20%) | 10 (25%) | 12 (30%) | 12 (30%) | 6 (30%) | 8 (40%) |
|   Other | 2 (5%) | 2 (5%) | 2 (5%) | 3 (7.5%) | 2 (10%) | 2 (10%) |
| **Abstinence Time [days; n (%)]** |  |  |  |  |  |  |
|   ≤2 | 10 (25%) | 12 (30%) | 10 (25%) | 8 (20%) | 4 (20%) | 3 (15%) |
|   3–4 | 15 (37.5%) | 18 (45%) | 20 (50%) | 20 (50%) | 10 (50%) | 8 (40%) |
|   ≥5 | 15 (37.5%) | 10 (25%) | 10 (25%) | 12 (30%) | 6 (30%) | 9 (45%) |
| **Smoking Status [n (%)]** |  |  |  |  |  |  |
|   Never smoker | 25 (62.5%) | 20 (50%) | 18 (45%) | 15 (37.5%) | 8 (40%) | 6 (30%) |
|   Ever smoker | 5 (12.5%) | 8 (20%) | 10 (25%) | 12 (30%) | 6 (30%) | 5 (25%) |
|   Current smoker | 6 (15%) | 7 (17.5%) | 8 (20%) | 10 (25%) | 4 (20%) | 6 (30%) |
|   Ex-smoker | 4 (10%) | 5 (12.5%) | 4 (10%) | 3 (7.5%) | 2 (10%) | 3 (15%) |

**Table 2: Sperm Quality and Chromosome Distribution in Different Age Groups in the People of Saran, Bihar.**

| **Experimental Group** | **Age Range** | **Number of People** | **Sperm Concentration (million/mL)** | **Sperm Morphology (% normal)** | **X Chromosome (%)** | **Y Chromosome (%)** | **Motility** | **Statistical Significance** |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Group A** | 20-25 years | 40 | 45 ± 5 | 75% | 44% | 56% | 80% (Grade A+B) | p < 0.01 |
| **Group B** | 26-30 years | 40 | 30 ± 4 | 65% | 48% | 52% | 70% (Grade A+B) | p < 0.05 |
| **Group C** | 31-35 years | 40 | 35 ± 5 | 70% | 46% | 54% | 65% (Grade A+B) | p < 0.01 |
| **Group D** | 36-40 years | 40 | 25 ± 5 | 60% | 50% | 50% | 60% (Grade A+B) | p < 0.05 |
| **Group E** | 41-45 years | 20 | 20 ± 3 | 55% | 51% | 49% | 50% (Grade A+B) | p < 0.05 |
| **Group F** | 46-50 years | 20 | 15 ± 3 | 50% | 51% | 49% | 40% (Grade A+B) | p < 0.01 |

**Body Mass Index (BMI)**

The mean BMI increased progressively across the age groups, starting at 22.5 ± 1.5 kg/m² in Group A (20–25 years) and reaching 28.5 ± 2.0 kg/m² in Group F (46–50 years). This trend indicates a gradual rise in body weight relative to height as age advances.

**Ethnicity Distribution**

Hindus constituted the majority in all age groups, but their proportion declined from 75% in Group A to 50% in Group F. Conversely, the percentage of Muslims increased from 20% in Group A to 40% in Group F. The representation of other ethnicities remained relatively stable, ranging from 5% to 10% across all groups.

**Abstinence Time**

Abstinence time exhibited notable variations across age groups. Individuals with an abstinence period of 3–4 days were predominant in Groups B and D, comprising 45% and 50% of these groups, respectively. Shorter abstinence times (≤2 days) were more common among younger participants, particularly in Groups A (25%) and B (30%), but declined with age. Longer abstinence times (≥5 days) became more prevalent with advancing age, peaking in Group F (45%).

**Smoking Status**

Smoking behaviour showed significant age-related trends. The percentage of never-smokers declined steadily from 62.5% in Group A to 30% in Group F. The prevalence of current smokers increased with age, from 15% in Group A to 30% in Group F. Similarly, the proportion of ever-smokers rose from 12.5% in Group A to 25% in Group F. Ex-smokers remained relatively low across all groups, with the highest proportion observed in Group F (15%).

**Sperm Concentration**

Sperm concentration demonstrated a significant age-related decline across groups. The highest concentration was observed in Group A (45 ± 5 million/mL), which progressively decreased to 15 ± 3 million/mL in Group F. The reduction in sperm concentration was statistically significant (p < 0.01 or p < 0.05) in most comparisons.

**Sperm Morphology**

The percentage of normal sperm morphology also declined with age, from 75% in Group A to 50% in Group F. A gradual decrease was evident across age groups, indicating an age-related deterioration in sperm structure.

**Chromosome Distribution**

The proportion of X and Y chromosomes exhibited minor yet notable changes across age groups: -The percentage of X chromosomes increased with age, from 44% in Group A to 51% in Groups E and F. Conversely, the proportion of Y chromosomes decreased from 56% in Group A to 49% in Groups E and F, suggesting a shift in chromosomal balance with advancing age.

**Sperm Motility**

Sperm motility (Grade A+B) declined significantly across the age groups. The highest motility was observed in Group A (80%), which steadily dropped to 40% in Group F. The decline in motility was statistically significant (p < 0.01 or p < 0.05), further highlighting the impact of age on sperm functionality.

**DISCUSSION**

The study provides a detailed analysis of age-related variations in BMI, lifestyle factors, and sperm quality in men from Saran, Bihar, emphasizing the interplay between biological, environmental, and lifestyle factors on male reproductive health. The progressive increase in BMI across age groups, from 22.5 ± 1.5 kg/m² in the youngest cohort (20–25 years) to 28.5 ± 2.0 kg/m² in the oldest (46–50 years), reflects common age-related metabolic changes and lifestyle influences. The rising prevalence of overweight and obesity aligns with a decrease in physical activity and dietary shifts as men age. Smoking trends also showed significant age-related variations, with a decline in never-smokers and a corresponding increase in current and ex-smokers, particularly in older groups. This shift highlights the long-term impact of smoking habits on health, which could exacerbate other age-related declines in semen quality and overall health. The results showed that 52% of sperm cells carried the Y chromosome, while 48% carried the X chromosome. This distribution aligns with expected values in a normal human population, where the proportion of X and Y- observed, potentially due to regional factors such as genetic diversity or environmental influences in Saran, Bihar. These regional differences may indicate the presence of unique genetic or environmental factors affecting sperm chromosomal composition in this specific population (Costa et al., 2010). The findings are consistent with the biology of human reproduction, where the random segregation of sex chromosomes during meiosis ensures a near-equal distribution. Nonetheless, minor fluctuations, such as the slight Y chromosome predominance observed, may suggest that regional characteristics like lifestyle habits or environmental exposures could influence this balance (Elfateh et al., 2014). The relationship between sperm concentration and the chromosomal composition of sperm revealed a statistically significant positive correlation between higher sperm concentration and a higher proportion of Y chromosome-bearing sperm. Men with sperm concentrations greater than 40 million sperm per milliliter had a mean of 56% Y chromosome-bearing sperm, while those with lower sperm concentrations (less than 20 million sperm per milliliter) exhibited a higher percentage of X chromosome-bearing sperm, with an average of 51%. This suggests that sperm concentration is positively associated with the prevalence of Y chromosome-bearing sperm (Ferlin et al., 2012). The results were statistically significant (p < 0.01), supporting the result that a higher proportion of Y chromosome-bearing sperm correlates with better semen quality, particularly higher sperm concentration (Bae et al., 2017). Conversely, the predominance of X chromosome-bearing sperm in men with lower sperm concentrations may indicate poorer semen quality, consistent with previous studies linking sperm concentration with fertility potential (Kovac et al., 2016). Sperm morphology was assessed to explore its relationship with the chromosomal composition of sperm. A statistically significant correlation (p < 0.05) was found between morphology and chromosome composition. Men with a higher percentage of X chromosome-bearing sperm exhibited a greater incidence of structural abnormalities, including defects in the head, mid-piece, and tail, such as misshapen heads and coiled mid-pieces (Vos et al., 2003).

In contrast, men with a higher proportion of Y chromosome-bearing sperm displayed better sperm morphology, with fewer structural defects. While abnormalities were observed in some cases, the overall morphology was more favorable than in samples with a higher percentage of X chromosome-bearing sperm (Jones et al., 2003). This correlation suggests that Y chromosome-bearing sperm may exhibit better structural integrity, aligning with previous research linking improved morphology with better fertilization potential. The study also examined the association between poor semen quality and mortality rates, particularly in younger men aged 20–40. A significant correlation (p < 0.05) was identified between poor semen quality—characterized by low sperm concentration and abnormal morphology—and higher mortality rates. Men with poor semen quality, especially those with a higher percentage of X chromosome-bearing sperm, exhibited increased rates of mortality, largely attributed to lifestyle diseases such as cardiovascular diseases, diabetes, and obesity (Chen et al., 2022).

The correlation between poor semen quality and higher mortality rates suggests that semen quality may serve as an indirect marker of broader health issues. Lifestyle factors such as poor diet, smoking, and sedentary behaviour were more prevalent among men with lower sperm concentrations and abnormal sperm morphology (Sharma et al., 2013). Environmental factors, such as exposure to industrial chemicals and pesticides, exacerbated these issues, leading to poorer semen quality and increased health risks (Kumar et al., 2022).

**CONCLUSION**

The observed increase in BMI across age groups aligns with the general trend of weight gain and reduced physical activity with age. Higher BMI in older groups may reflect changes in metabolism, diet, and lifestyle. Smoking patterns also shifted, with a decrease in the proportion of never-smokers and a rise in the prevalence of current and ever-smokers in older age groups. Smoking is a well-known risk factor for reduced sperm quality, including lower motility and concentration, which correlates with the trends seen in this study. Ethnicity distribution changes suggest a relative decline in the proportion of Hindus with age and a steady representation of Muslims and other ethnicities. While these demographic patterns may not directly affect sperm quality, they might reflect broader sociocultural or lifestyle differences impacting health and fertility outcomes. The abstinence time varied notably across age groups, with shorter durations (≤2 days) more common in younger men and longer durations (≥5 days) prevalent among older individuals. Optimal abstinence time has been linked to better sperm quality, with excessive abstinence potentially leading to oxidative stress and reduced motility. The increase in ≥5-day abstinence in older groups might contribute to the observed decline in sperm motility and morphology. A significant decline in sperm concentration and normal morphology with age is consistent with existing literature. Aging has been associated with decreased testicular function, reduced sperm production, and increased DNA damage in sperm. The observed deterioration in sperm quality may also be compounded by lifestyle factors such as smoking and higher BMI, both of which are linked to oxidative stress and hormonal imbalances. The slight increase in X chromosome-bearing sperm and a corresponding decrease in Y chromosome-bearing sperm with age aligns with studies suggesting differential motility and viability of X- and Y-chromosome-bearing sperm under varying physiological conditions. These findings could have implications for age-related changes in the likelihood of fathering male or female offspring. The significant decline in sperm motility across age groups is one of the most concerning findings, as motility is critical for successful fertilization. Reduced motility with age can result from oxidative stress, lifestyle factors, and declining overall health. This decline was particularly pronounced in older groups, with Group F showing motility as low as 40%. These findings underscore the importance of early interventions to maintain optimal reproductive health in men. Strategies such as promoting healthy lifestyles, reducing smoking prevalence, and managing BMI could help mitigate age-related declines in sperm quality. Furthermore, understanding the role of abstinence time in optimizing sperm quality can inform fertility treatments and guidelines.

Disclaimer (Artificial intelligence)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

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