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Assessment Of Sperm DNA Fragmentation And Hormonal Alterations In Agrochemical-Exposed Farmers Of Dhamtari District, Chhattisgarh

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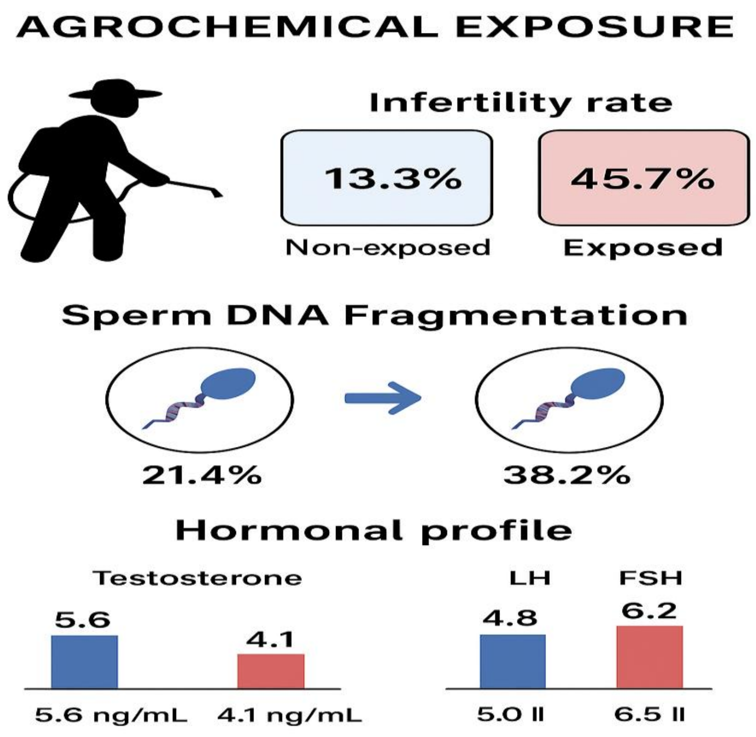
Abstract
Chronic exposure to agrochemicals is a major occupational hazard in agricultural communities, potentially affecting male fertility through oxidative stress and endocrine disruption.To evaluate the effect of agrochemical exposure on infertility rates, sperm DNA fragmentation, and key reproductive hormones in farmers of Dhamtari district, Chhattisgarh.A total of 100 male farmers were enrolled: 70 with regular agrochemical exposure (exposed group) and 30 without such exposure (non-exposed group). Semen analysis, sperm DNA fragmentation assay, and serum hormonal profiling (testosterone, luteinizing hormone [LH], follicle-stimulating hormone [FSH]) were performed. Infertility was defined by WHO criteria. Statistical comparisons were made using independent t-tests and Chi-square tests.Infertility rate was significantly higher in the exposed group (45.7%) than in the non-exposed group (13.3%) (χ^2 = 10.92, $p < 0.01$). Mean sperm DNA fragmentation was elevated in exposed farmers (38.2 ± 6.5%) compared to non-exposed (21.4 ± 5.1%) ($p < 0.001$). Testosterone levels were significantly lower in exposed farmers (4.1 ± 0.7 ng/mL vs. 5.6 ± 0.8 ng/mL, $p < 0.001$), while LH and FSH were significantly higher ($p < 0.001$). Chi-square analysis confirmed significant associations between agrochemical exposure and high DNA fragmentation, low testosterone, and altered gonadotropin levels.Agrochemical exposure is strongly associated with increased infertility risk, elevated sperm DNA fragmentation, and hormonal dysregulation in male farmers. These findings highlight the urgent need for occupational health interventions and safer agricultural practices.
Keywords: *agrochemicals, infertility, DNA fragmentation index, hormones, farmers, pesticides*

Graphical Abstract:



INTRODUCTION: Agricultural development has been the cornerstone of food security and rural livelihoods in India. However, this progress has also brought a significant dependence on agrochemicals such as pesticides, herbicides, fungicides, and synthetic fertilizers to boost crop yield and manage pest infestations (7). While these substances have improved agricultural productivity, they pose a hidden and growing threat to human health—especially to farmers and agricultural laborers who are occupationally exposed. Among the lesser-discussed consequences of chronic agrochemical exposure is its adverse impact on male reproductive health, including hormonal imbalances and sperm DNA integrity.India, one of the largest users of agrochemicals in the developing world, has reported increasing cases of infertility, particularly in agricultural regions (16). Studies suggest that farmers who are routinely exposed to a cocktail of pesticides and other chemicals during spraying, mixing, or storage are at higher risk of reproductive dysfunction (10). These substances may act as endocrine-disrupting chemicals (EDCs), interfering with hormonal regulation and affecting spermatogenesis. In particular, exposure to organophosphates, carbamates, pyrethroids, and organochlorines has been linked to reduced sperm quality, altered sperm morphology, suppressed testosterone levels, and even damage to the genetic material carried in spermatozoa (9).

Sperm DNA fragmentation (SDF) is now recognized as a key biomarker of male infertility and reproductive competence. While standard semen parameters (sperm count, motility, morphology) remain important, they do not fully reflect functional integrity. DNA fragmentation tests such as the TUNEL assay or Comet assay can detect single- and double-strand breaks in sperm DNA—critical for fertilization, embryo development, and the health of offspring (11). Numerous studies have linked elevated levels of sperm DNA fragmentation to miscarriages, failed assisted reproductive technologies (ART), and poor embryo quality. Importantly, DNA damage in sperm is often caused by oxidative stress, which is one of the known outcomes of pesticide exposure (12).

Hormones such as follicle-stimulating hormone (FSH), luteinizing hormone (LH), and testosterone play vital roles in male fertility. FSH is essential for spermatogenesis by acting on Sertoli cells, LH stimulates Leydig cells to produce testosterone, and testosterone regulates libido, sperm production, and secondary sexual characteristics (17). Disruption of this hormonal axis due to exposure to environmental toxins can result in hypo- or hypergonadotropic states, reduced sperm production, and erectile dysfunction. Several pesticides are known to mimic or block hormone receptors or interfere with hormone synthesis pathways, resulting in systemic endocrine disruption (8).

The Dhamtari district of Chhattisgarh is one of the agriculturally intensive zones in central India, where paddy cultivation dominates the agrarian landscape. Farmers in this region routinely use various agrochemicals with minimal personal protective equipment or safety training. Long-term and cumulative exposure to these chemicals raises concerns about subclinical and clinical reproductive health issues in this vulnerable group (18). Despite this, there is limited region-specific research that systematically evaluates the extent to which occupational agrochemical exposure affects sperm DNA integrity and hormonal balance (1).

This study was designed to bridge that gap. We investigated and compared the sperm DNA fragmentation index and hormonal profiles (testosterone, LH, and FSH) of 230 agrochemical-exposed male farmers with 30 non-exposed individuals from the same region. The primary objective was to determine whether chronic exposure to agrochemicals is associated with a statistically significant increase in sperm DNA damage and alterations in key reproductive hormones (2). We also assessed the correlation between DNA fragmentation and infertility status to evaluate the reproductive implications of such exposure (5, 23).By focusing on a homogenous rural population with well-defined exposure history and including both infertile and fertile individuals, this study aims to contribute meaningful evidence to the growing body of literature on environmental reproductive toxicology (24). In doing so, it highlights the urgent need for public health interventions, farmer education, and regulatory control of agrochemical use. Additionally, our findings may inform fertility clinics and reproductive specialists about the relevance of environmental exposure histories in diagnosing and treating male infertility (6).

Given the increasing global concern over reproductive health disorders and declining sperm quality, this study is both timely and necessary. It reinforces the need to consider environmental and occupational exposures in the broader discussion of male fertility decline—a trend that is often attributed solely to lifestyle factors or genetics (13). Through this regional case study, we hope to raise awareness about the often-overlooked reproductive consequences of agrochemical exposure and advocate for improved monitoring and preventive strategies in rural India (4).

MATERIALS AND METHODS

2.1 Study Design and Population

A cross-sectional study was conducted among 100 male farmers from the Dhamtari district, Chhattisgarh. The exposed group (n = 70) comprised farmers with ≥5 years of direct agrochemical exposure during farming activities. The non-exposed group (n = 30) included farmers with no reported occupational agrochemical contact. (3).

2.2 Inclusion and Exclusion criteria

Inclusion criteria represent males aged 21–50 years, married, actively trying for conception. Exclusion criteria represent history of varicocele, cryptorchidism, systemic illness, or recent febrile episode. (14).

2.3 Semen Sample Collection

Samples were collected by masturbation following 2–5 days of abstinence and allowed to liquefy at 37 °C. Basic semen parameters were recorded prior to fragmentation testing (19).

2.4 DNA Fragmentation Analysis (SCD Test)

DNA fragmentation was measured using the QwikCheck™ DFI kit. At least 200 spermatozoa per sample were analyzed. Sperm showing large/medium halos were classified as non-fragmented; those with small/no halos or degeneration were considered fragmented. Following are the standard interpretation:<15% fragmentation: Excellent fertility potential, 15–30%: Fair to good fertility, 30%: Poor fertility potential (20).

2.5 Hormonal Assessment

Blood samples were collected to evaluate serum testosterone, FSH, and LH levels using electrochemiluminescence assays. Reference values used:Testosterone: 249–1080 ng/dl, LH: 1.5–9.30 mIU/ml, FSH: 1.4–18.11 mIU/ml (15).

2.6 Statistical Analysis

Categorical data were analyzed using Chi-square tests to evaluate associations between exposure and infertility, DFI, and hormone levels. A p-value <0.05 was considered statistically significant. Analyses were performed using SPSS software (21, 15).

3. RESULTS

3.1 Agrochemical Usage and Fertility

A Significant difference in infertility rates was observed between agrochemical exposed and non-exposed in (Table 1). Among the non-exposed group, only 13.3% (4 out of 30) were infertile, compared to 45.7% (32 out of 70) in the exposed group, indicating a more than threefold higher infertility rate among those exposed to agrochemicals.

Table 1. Agrochemical exposure and infertility rate

Group	Infertile (n)	Fertile (n)	Total (n)	Infertility Rate (%)
Non-exposed	4	26	30	13.3%
Exposed	32	38	70	45.7%

3.2 Sperm DNA Fragmentation and Hormonal analysis

Mean sperm DNA fragmentation percentage was significantly higher in the exposed group (38.2 ± 6.5%) compared to the non-exposed group (21.4 ± 5.1%; $p < 0.001$) (Table 2). Testosterone levels were significantly lower in exposed farmers (4.1 ± 0.7 ng/mL) than in non-exposed farmers (5.6 ± 0.8 ng/mL; $p < 0.001$).

Similarly, serum LH and FSH levels were significantly elevated in the exposed group ($p < 0.001$ for both), suggesting possible hypothalamic-pituitary-gonadal axis dysregulation associated with agrochemical exposure.

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Table 2. Sperm DNA fragmentation and hormonal profile

Parameter	Non-exposed (n=30)	Exposed (n=70)	p-value
DNA Fragmentation (%)	21.4 ± 5.1	38.2 ± 6.5	<0.001
Testosterone (ng/mL)	5.6 ± 0.8	4.1 ± 0.7	<0.001
LH (mIU/mL)	4.8 ± 0.9	6.2 ± 1.0	<0.001
FSH (mIU/mL)	5.0 ± 0.8	6.5 ± 0.9	<0.001

3.3 Chi- square analysis

Chi-square tests confirmed statistically significant associations between agrochemical exposure and all measured reproductive health outcomes (Table 3). Infertility was strongly associated with exposure (χ^2 = 10.92, p = 0.0009). High DNA fragmentation (>30%) was significantly more prevalent in exposed farmers (χ^2 = 15.37, p = 0.00008).

Similarly, low testosterone, abnormal LH, and abnormal FSH levels were all significantly associated with agrochemical exposure (p< 0.05 for each).

Table 3. Chi-square analysis of categorical reproductive health outcomes

Parameter	Exposed (n=70)	Non-exposed (n=30)	χ^2 Value	p-value	Significance
Infertility rate (%)	32 (45.7%)	4 (13.3%)	10.92	0.0009	p<0.01
High DNA fragmentation (%)	42 (60%)	6 (20%)	15.37	0.00008	p<0.001
Low testosterone (%)	29 (41.4%)	4 (13.3%)	8.21	0.0042	p<0.01
Low LH (%)	25 (35.7%)	3 (10%)	6.48	0.0109	p<0.05
Low FSH (%)	27 (38.6%)	4 (13.3%)	7.12	0.0076	p<0.01

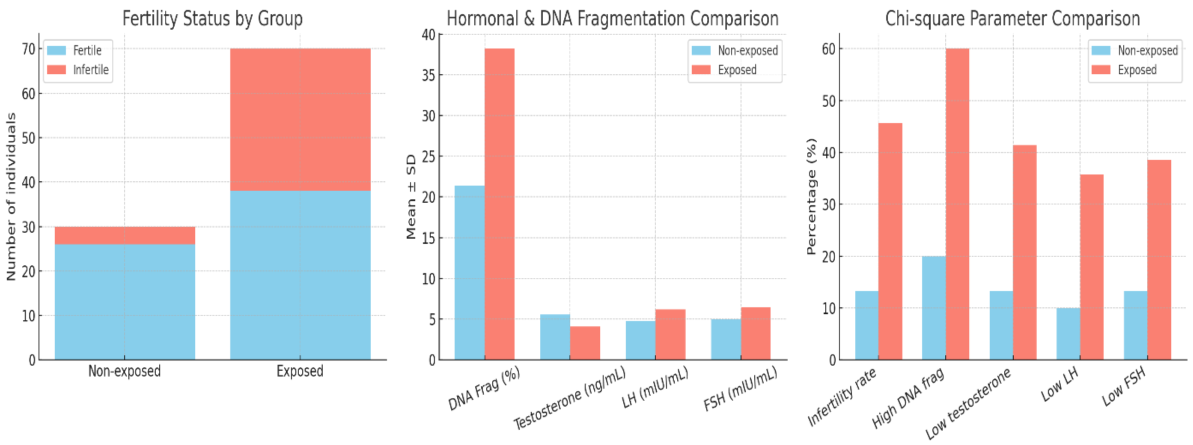

Figure 1. Comparative analysis of agrochemical exposure effects on male reproductive parameters: (A) Fertility status distribution between exposed and non-exposed groups. (B) Mean sperm DNA fragmentation and hormone levels (Testosterone, LH, FSH) in both groups.

DISCUSSION: Our findings demonstrate a clear association between agrochemical exposure and adverse reproductive outcomes in male farmers. The infertility rate among exposed individuals (45.7%) was more than threefold higher than in the non-exposed group (13.3%), aligning with earlier reports linking pesticide exposure to impaired spermatogenesis and conception delay.

Elevated DNA fragmentation in exposed farmers suggests that oxidative stress and genotoxic effects are critical pathways of agrochemical-induced reproductive toxicity. Similar observations have been made in studies where organophosphates and carbamates were associated with increased reactive oxygen species (ROS) generation and compromised sperm chromatin integrity.

Testosterone levels were significantly lower in the exposed group, indicating possible Leydig cell dysfunction or hypothalamic-pituitary-gonadal axis disruption. In contrast, LH and FSH levels were higher, potentially reflecting a compensatory pituitary response to primary testicular failure. This endocrine pattern is consistent with subfertility profiles reported in other agricultural populations.

Chi-square analysis reinforced the strength of these associations, with significant differences in infertility rate, DNA fragmentation, and hormonal parameters between groups. The findings underscore the multifactorial nature of agrochemical-induced male infertility, involving direct gonadotoxicity, endocrine disruption, and oxidative DNA damage.

Given the occupational nature of exposure, preventive interventions such as use of personal protective equipment (PPE), safer handling practices, and substitution with less toxic agrochemicals are urgently warranted. Regular reproductive health screening in high-risk agricultural communities may also aid early detection and management.

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