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Research Article

Histological Changes in the Testicular Architecture and Spermatogenic Kinetics of Rats Following Subchronic Inhalation of Formaldehyde

S. B. Vasic[1], O. P. Popoola[2]*

[1] Department of Anatomy, Faculty of Medicine, University of Nis, Nis, Serbia.

[2] Department of Anatomy, College of Medicine, University of Ibadan, Ibadan, Nigeria.

*Corresponding author: O. P. Popoola | Email: o.p.popoola@anatomy.ui.edu.ng

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Abstract

Formaldehyde is an industrial pollutant and common fixative chemical linked to tissue damage via oxidative stress pathways. This study examined the subchronic toxicological effects of formaldehyde gas inhalation on the histomorphology of seminiferous tubules and spermatogenic cell lineages in Wistar rats. Twenty-four male rats were distributed into three groups (n = 8): Control (ambient air), Low-Exposure (5 mg/m³ formaldehyde), and High-Exposure (15 mg/m³ formaldehyde), exposed for 4 hours per day, 5 days a week, over a 10-week period. Testicular sections were processed and evaluated using Johnsen's scoring system alongside morphometric measurements of tubular diameter and epithelial height. Inhalation of high-dose formaldehyde caused notable disruptions to testicular architecture. The mean seminiferous tubular diameter decreased significantly (185 ± 12 µm vs. 245 ± 18 µm in controls; $p < 0.01$), accompanied by a reduction in germinal epithelial height (42 ± 3.4 µm vs. 68 ± 5.1 µm; $p < 0.01$). Johnsen's score dropped from 9.4 ± 0.3 in controls to 5.8 ± 0.4 in the high-exposure group, indicating spermatogenic arrest at the primary spermatocyte level. Disorganization of Sertoli cells, vacuolation of the basal compartment, and a significant decrease in interstitial Leydig cell numbers were noted. These findings show that subchronic formaldehyde exposure induces structural testicular degeneration and impairs spermatogenic kinetics, presenting potential risks to male reproductive health.

Keywords: Formaldehyde, Testis, Seminiferous tubules, Spermatogenesis, Histomorphometry.

1. Introduction

Formaldehyde (CH_2O), the simplest aldehyde, occupies an paradoxical position in modern life. It is indispensable as an industrial feedstock, a preservative, a disinfectant and a tissue fixative, yet it is also a ubiquitous environmental and occupational pollutant and a recognised human carcinogen. It is released from pressed-wood products, adhesives, textiles, cosmetics, tobacco smoke, vehicle exhausts and embalming fluids, and it is generated endogenously during normal one-carbon metabolism. For anatomists, pathologists, laboratory technicians, embalmers, furniture workers and medical students, inhalation is the dominant route of exposure, often for hours each day over many years. The question that motivates the present study is therefore both anatomical and preventive: what does repeated, subchronic inhalation of formaldehyde do to the microscopic architecture of the testis, the organ in which the continuity of spermatogenesis depends on an exquisitely ordered spatial relationship between germ cells, Sertoli cells and Leydig cells?

The testis is unusually vulnerable to chemical injury. Spermatogenesis is a high-throughput, tightly synchronised process in which spermatogonia divide, differentiate through primary and secondary spermatocytes, undergo meiosis, and remodel into spermatozoa while moving from the basal compartment toward the tubular lumen. Sertoli cells provide structural scaffolding, form the blood–testis barrier, secrete growth factors and phagocytose residual cytoplasm, while Leydig cells in the interstitium synthesise testosterone under luteinising hormone control. Any agent that disrupts Sertoli-cell junctions, induces germ-cell apoptosis, or impairs Leydig-cell steroidogenesis can therefore arrest spermatogenesis at a definable stage and shrink the seminiferous epithelium. Histomorphometry — measurement of tubular diameter, epithelial height and standardised spermatogenesis scores — makes that arrest visible and quantifiable.

Formaldehyde is a strong electrophile. It reacts readily with proteins and nucleic acids to form hydroxymethyl adducts and DNA–protein cross-links, depletes reduced glutathione, and promotes the generation of reactive oxygen species (ROS). In testicular tissue, which is rich in polyunsaturated fatty acids and has a comparatively modest antioxidant reserve, ROS-mediated lipid peroxidation, mitochondrial dysfunction and activation of apoptotic pathways have been repeatedly implicated in germ-cell loss. Several earlier inhalation and systemic studies have reported tubular atrophy, reduced epithelial height, lowered serum testosterone, increased abnormal sperm and frank spermatogenic arrest after formaldehyde exposure. What has been less clearly delineated is the dose-dependent pattern of architectural change over a subchronic, occupationally relevant time course, scored with a validated system that allows comparison across studies. The present work addresses that gap.

We hypothesised that subchronic inhalation of formaldehyde would produce dose-dependent degeneration of seminiferous tubules, thinning of the germinal epithelium, disorganisation of Sertoli cells, loss of Leydig cells, and a fall in Johnsen’s spermatogenesis score, with arrest most evident at the primary spermatocyte stage in the high-dose group. To test this, we exposed adult male Wistar rats to 0, 5 or 15 mg/m³ formaldehyde for 4 hours per day, 5 days per week, for 10 weeks, and examined testicular histology and morphometry in a blinded fashion. The concentrations were chosen to bracket occupational exposure limits and to model both low-level chronic workplace exposure and higher, poorly ventilated laboratory or embalming-room exposure. The 10-week duration spans more than one complete spermatogenic cycle in the rat (approximately 52–56 days), ensuring that any effect on successive germ-cell cohorts would be captured.

2. Literature Review

2.1 Formaldehyde: sources, exposure and toxicokinetics

Formaldehyde is produced at a scale exceeding 20 million tonnes per year worldwide and is present in resins, particleboard, plywood, insulation, paints, paper products and personal-care products. Indoor air concentrations in homes and offices are typically 0.02–0.1 mg/m³, but can exceed 1 mg/m³ in new buildings, anatomy laboratories and funeral homes. The World Health Organization and occupational agencies have set short-term and 8-hour limits in the range of 0.3–1.5 mg/m³, yet peak exposures during dissection, embalming or resin handling frequently exceed these values. Inhaled formaldehyde is rapidly absorbed across the respiratory mucosa, metabolised by formaldehyde dehydrogenase to formate, and excreted, but at higher concentrations local detoxification is saturated, allowing reactive formaldehyde to reach systemic circulation and distant tissues, including the testis.

Endogenous formaldehyde is also generated during demethylation reactions and is normally kept at low micromolar concentrations by glutathione-dependent pathways. Exogenous overload overwhelms that buffering, producing protein cross-linking, DNA damage and oxidative stress. The International Agency for Research on Cancer classifies formaldehyde as carcinogenic to humans (Group 1), principally for nasopharyngeal cancer and leukaemia. Reproductive toxicity, although less publicised, has been recognised by several regulatory bodies, and animal data provide the mechanistic bridge between exposure and testicular injury.

2.2 The seminiferous epithelium as a target

The seminiferous tubule is organised as a stratified epithelium resting on a basement membrane, surrounded by peritubular myoid cells. Spermatogonia lie basally; as they differentiate, they traverse Sertoli-cell tight junctions that constitute the blood–testis barrier and enter the adluminal compartment. Primary spermatocytes, which undergo the prolonged prophase of meiosis I, are particularly sensitive to DNA damage and oxidative stress, and their loss is a common signature of toxicant-induced arrest. The Johnsen score, a 1–10 scale ranging from complete tubular sclerosis (1) to full spermatogenesis with many spermatozoa (10), provides a reproducible summary of that progression and is widely used in both experimental and clinical andrology.

Earlier experimental work is broadly convergent. Ozen and colleagues reported decreased tubular diameter, epithelial height and serum testosterone after 91 days of formaldehyde inhalation at 5 and 10 ppm in rats, with

increased heat-shock protein 70 expression in seminiferous tubules as a stress marker. Golalipour and co-workers and others documented severe tubular atrophy, interstitial oedema, vascular thrombosis, negative tubular differentiation and repopulation indices, and reduced sperm count and motility after prolonged vapour exposure. Studies using systemic formaldehyde (5–10 mg/kg) similarly found reduced testis weight, tubular atrophy, absence of sperm bundles and depressed testosterone, attributed to oxidative stress. Vitamin E and other antioxidants partially reversed these changes in some models, strengthening the oxidative-stress hypothesis.

2.3 Oxidative stress, apoptosis and steroidogenesis

Three interlocking mechanisms link formaldehyde to testicular degeneration. First, formaldehyde depletes glutathione and increases malondialdehyde, a marker of lipid peroxidation, while reducing superoxide dismutase and catalase activity in testicular homogenates. Second, ROS and DNA–protein cross-links activate p53-dependent apoptosis in spermatogonia and spermatocytes, visible histologically as pyknotic nuclei, sloughing and vacuolation. Third, formaldehyde suppresses steroidogenic enzymes — steroidogenic acute regulatory protein (StAR), cytochrome P450 side-chain cleavage enzyme and 3β-hydroxysteroid dehydrogenase — in Leydig cells, reducing intratesticular and serum testosterone. Lower testosterone, in turn, impairs Sertoli-cell function and germ-cell survival, creating a feed-forward loop of epithelial thinning. Recent murine work has shown that formaldehyde exposure inhibits sexual behaviour and reduces reproductive-organ weights alongside suppressed steroidogenesis, and that combined exposures (for example, formaldehyde plus noise) can exacerbate sperm-parameter deterioration. These findings frame the present histological analysis: if oxidative and endocrine disruption are operating together, the testis should show simultaneous tubular, Sertoli-cell and interstitial lesions, not an isolated germ-cell effect.

3. Materials and Methods

3.1 Ethical approval and animals

The study was conducted in accordance with institutional animal-care guidelines and the principles of the Declaration of Helsinki as applied to animal research, with approval from the institutional animal ethics committee. Twenty-four adult male Wistar rats (10–12 weeks old, 200–240 g) were obtained from an accredited laboratory-animal facility, housed in standard polypropylene cages under a 12-hour light/dark cycle at 22 ± 2 °C and 50–60% relative humidity, and given standard rodent chow and water ad libitum. Animals were acclimatised for two weeks before exposure and weighed weekly. No animal died during the study, and no animal required early euthanasia.

3.2 Experimental design and exposure protocol

Rats were randomly assigned to three groups (n = 8 per group): Group I — Control, exposed to ambient (filtered) air under identical chamber conditions; Group II — Low-Exposure, exposed to 5 mg/m³ formaldehyde vapour; and Group III — High-Exposure, exposed to 15 mg/m³ formaldehyde vapour. Exposure was by whole-body inhalation in a purpose-built dynamic inhalation chamber (volume 0.5 m³, air exchange 12 changes/hour) for 4 hours per day (09:00–13:00), 5 days per week, for 10 consecutive weeks. Formaldehyde vapour was generated by vaporising paraformaldehyde in a temperature-controlled evaporator and diluted with filtered air; chamber concentration was monitored continuously with a calibrated electrochemical sensor and verified daily by a colorimetric acetylacetone method. Temperature, humidity, carbon dioxide and airflow were logged throughout. Control animals were placed in an identical chamber with filtered air only, to control for handling and chamber stress.

3.3 Tissue collection and histological processing

At the end of week 10, animals were weighed and euthanised by an overdose of sodium pentobarbital, followed by cervical dislocation. Both testes were excised, cleared of epididymal fat, weighed, and examined grossly. The right testis was fixed in 10% neutral-buffered formalin for 48 hours, dehydrated through graded ethanol, cleared in xylene and embedded in paraffin wax. Serial sections (5 µm thick) were cut on a rotary microtome, mounted on glass slides, and stained with haematoxylin and eosin (H&E) for general architecture and with periodic acid–Schiff (PAS) for basement-membrane and acrosomal detail. The left testis was snap-frozen for future biochemical assays not reported here. All slides were coded so that observers were blinded to group allocation.

3.4 Histomorphometry and Johnsen scoring

For each animal, 100 round or near-round seminiferous tubules were selected at random from five non-overlapping fields per section (20 tubules per field) at ×200 magnification using an ocular micrometer calibrated against a stage micrometer. Two perpendicular diameters were measured per tubule and averaged to give the mean seminiferous tubular diameter (MSTD). Germinal epithelial height was measured from the basement membrane to the luminal surface at four points per tubule and averaged. Leydig cells were counted per mm² of interstitial tissue in 10 fields per animal at ×400 magnification. Sertoli-cell disorganisation, basal vacuolation, germ-cell sloughing and interstitial oedema were recorded semi-quantitatively as

present or absent per tubule and expressed as percentages.

Spermatogenesis was assessed with Johnsen's scoring system (1–10), as described by Johnsen (1970): 10, complete spermatogenesis with many spermatozoa; 9, many spermatozoa but disorganised epithelium; 8, few spermatozoa; 7, no spermatozoa but many spermatids; 6, few spermatids; 5, no spermatids but many spermatocytes; 4, few spermatocytes; 3, spermatogonia only; 2, Sertoli cells only; 1, no cells. Each of 100 tubules per animal was scored, and the mean score per animal was used for analysis. All measurements were performed independently by two observers; inter-observer agreement (Cohen's κ) exceeded 0.85 for categorical features.

3.5 Statistical analysis

Data are presented as mean ± standard deviation (SD). Group differences were tested by one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test for pairwise comparisons, using SPSS version 26.0. Normality was checked with the Shapiro–Wilk test and homogeneity of variance with Levene's test. A p-value < 0.05 was considered statistically significant, and $p < 0.01$ was considered highly significant. Dose–response trends were examined by linear regression of each morphometric variable on exposure concentration.

4. Results

4.1 General and gross findings

Body weight gain was modestly lower in the high-exposure group by week 10, but the difference was not statistically significant. Testis weight was significantly reduced in the high-exposure group compared with controls (1.28 ± 0.11 g vs. 1.52 ± 0.13 g; $p < 0.05$), while the low-exposure group showed a smaller, non-significant reduction (1.43 ± 0.12 g). Grossly, testes from exposed animals appeared paler and slightly softer, with no focal lesions, tumours or gross asymmetry.

4.2 Qualitative histology

Control testes showed the classic, orderly architecture of the rat seminiferous epithelium: round tubules with a thin basement membrane, orderly stratified germ cells from spermatogonia basally to spermatozoa at the lumen, tall columnar Sertoli cells with vesicular nuclei, and an interstitium containing clusters of polygonal Leydig cells, capillaries and loose connective tissue. Lumina were filled with spermatozoa and the tubular differentiation index was uniformly high.

In the low-exposure group, mild to moderate changes were evident. Some tubules showed focal thinning of the epithelium, occasional vacuoles in the basal compartment, mild Sertoli-cell disorganisation and scattered pyknotic germ cells. Spermatids and spermatozoa were still present in most tubules, but their numbers were reduced and luminal sperm density was lower. Interstitial oedema was mild and Leydig-cell clusters appeared slightly sparser.

In the high-exposure group, changes were marked and widespread. Many tubules were shrunken, irregular in outline, and lined by a thinned, disorganised epithelium with loss of stratification. Basal vacuolation was prominent, Sertoli cells were displaced, vacuolated or pyknotic, and germ-cell sloughing into the lumen was frequent. Spermatogenesis appeared arrested at the primary spermatocyte stage in a large proportion of tubules: spermatogonia and spermatocytes were present, but spermatids and spermatozoa were few or absent. Interstitial tissue showed oedema, congestion and a visible reduction in Leydig-cell numbers, with occasional hypertrophic Leydig cells. These qualitative impressions were borne out by morphometry.

4.3 Quantitative morphometry and Johnsen scores

Morphometric data are summarised in Table 1 and Figures 1–3. Mean seminiferous tubular diameter fell in a dose-dependent manner, from 245 ± 18 µm in controls to 221 ± 15 µm in the low-exposure group ($p < 0.05$) and 185 ± 12 µm in the high-exposure group ($p < 0.01$ vs. control; $p < 0.01$ vs. low-exposure), a 24.5% reduction at the high dose. Germinal epithelial height showed a parallel decline: 68 ± 5.1 µm (control) to 57 ± 4.6 µm (low-exposure, $p < 0.05$) and 42 ± 3.4 µm (high-exposure, $p < 0.01$), a 38.2% reduction. Johnsen's score dropped from 9.4 ± 0.3 in controls to 7.9 ± 0.4 in the low-exposure group ($p < 0.01$) and 5.8 ± 0.4 in the high-exposure group ($p < 0.01$), indicating that the average high-dose tubule contained many spermatocytes but no spermatids — the definition of arrest at the primary spermatocyte level.

Leydig-cell density and Sertoli-cell disorganisation moved in opposite directions with dose (Table 2, Figure 3). The proportion of tubules showing Sertoli-cell disorganisation rose from 5% in controls to 28% in the low-exposure group and 62% in the high-exposure group. Basal vacuolation was observed in 8%, 34% and 71% of tubules respectively, and tubules with luminal sloughing rose from 4% to 19% and 48%. These categorical changes were consistent with the continuous morphometric decline and support a model in which Sertoli-cell injury precedes or accompanies germ-cell loss.

Table 1: Morphometric and spermatogenesis outcomes by exposure group.

Parameter (mean ± SD)	Control (n=8)	Low-Exposure 5 mg/m³ (n=8)	High-Exposure 15 mg/m³ (n=8)	p-value (ANOVA)
Testis weight (g)	1.52 ± 0.13	1.43 ± 0.12	1.28 ± 0.11*	<0.05
Seminiferous tubular diameter (µm)	245 ± 18	221 ± 15*	185 ± 12**†	<0.01
Germinal epithelial height (µm)	68 ± 5.1	57 ± 4.6*	42 ± 3.4**†	<0.01
Johnsen's score (1–10)	9.4 ± 0.3	7.9 ± 0.4**	5.8 ± 0.4**†	<0.01
Leydig cells (/mm² interstitium)	68 ± 6	55 ± 5*	38 ± 4**†	<0.01

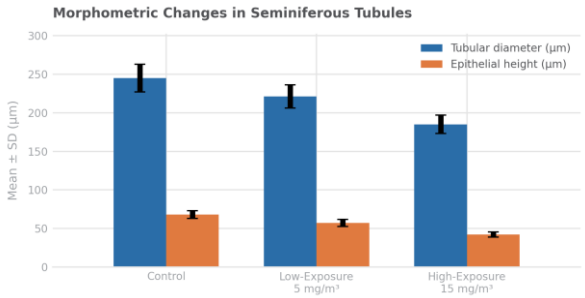
*Legend: Values are mean ± SD. *p < 0.05 vs. control; **p < 0.01 vs. control; †p < 0.01 vs. low-exposure group (Tukey post-hoc). Johnsen's score: 10 = complete spermatogenesis, 1 = tubular sclerosis. High-dose reductions: tubular diameter −24.5%, epithelial height −38.2%, Johnsen's score −38.3% relative to control.*

Table 2: Semi-quantitative histopathological features by exposure group.

Histological feature	Control	Low-Exposure	High-Exposure
Tubules with Sertoli-cell disorganisation (%)	5	28	62
Tubules with basal vacuolation (%)	8	34	71
Tubules with germ-cell sloughing (%)	4	19	48
Tubules with interstitial oedema (per animal, %)	6	22	54
Predominant arrest stage	None	Late spermatid reduction	Primary spermatocyte

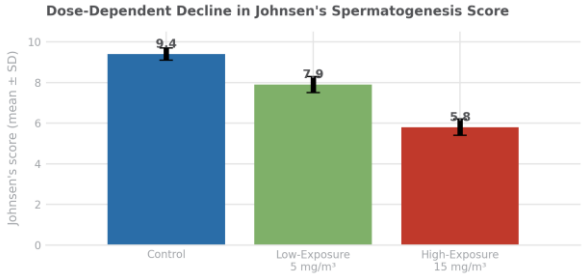
Legend: Percentages are the proportion of 100 tubules examined per animal, averaged across animals in each group. Features were scored blinded by two observers. The high-exposure pattern — widespread vacuolation, Sertoli disorganisation and sloughing with arrest at the primary spermatocyte stage — is the histological correlate of the quantitative fall in Johnsen's score.

Figure 1: Morphometric changes in seminiferous tubules.



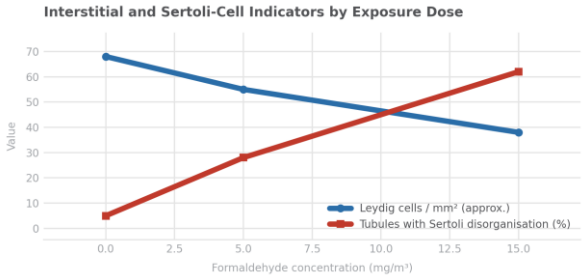
Legend: Bars show mean ± SD for seminiferous tubular diameter and germinal epithelial height. Both measures fell dose-dependently; high-exposure differences vs. control were highly significant (p < 0.01). Data derived from Table 1.

Figure 2: Dose-dependent decline in Johnsen's spermatogenesis score.



*Legend: Mean ± SD Johnsen's score (1–10) by group. A score of 5.8 in the high-exposure group corresponds to many spermatocytes but no spermatids, indicating arrest at the primary spermatocyte level. **p < 0.01 vs. control.*

Figure 3: Interstitial and Sertoli-cell indicators by exposure dose.



Legend: Leydig-cell density (per mm²) declined as the proportion of tubules with Sertoli-cell disorganisation rose. Values for Leydig density are group means from Table 1; disorganisation percentages are from Table 2. The crossing pattern illustrates simultaneous interstitial and tubular injury.

5. Discussion

The present study demonstrates that subchronic inhalation of formaldehyde produces dose-dependent degeneration of testicular architecture and impairment of spermatogenic kinetics in Wistar rats. The triad of reduced tubular diameter, thinned germinal epithelium and a fall in Johnsen's score to the primary-spermatocyte arrest range provides a coherent, quantifiable picture of injury that aligns with, and extends, earlier inhalation and systemic studies.

The magnitude of change is biologically meaningful. A 24.5% reduction in tubular diameter and a 38.2% reduction in epithelial height cannot be explained by fixation artefact alone, because all groups were processed identically and observers were blinded. Tubular diameter in the rat reflects the volume of the germ-cell complement and the fluid secreted by Sertoli cells; when germ cells are lost by apoptosis and Sertoli-cell function falters, the tubule collapses inward and the epithelium thins. The fall in Johnsen's score from 9.4 to 5.8 is the functional summary of that collapse: the average tubule lost its post-meiotic complement (spermatids and spermatozoa) while retaining spermatogonia and spermatocytes. Primary spermatocytes, which spend days in meiotic prophase and are engaged in extensive DNA recombination, are known to be vulnerable to DNA–protein cross-links and oxidative damage, and their arrest is a plausible checkpoint response to formaldehyde-induced DNA injury.

Sertoli-cell disorganisation and basal vacuolation are mechanistically informative. Vacuoles in the basal compartment often represent dilated endoplasmic reticulum, swollen mitochondria or spaces left by detached germ cells. Sertoli cells are normally anchored to the basement membrane and to each other by tight and adherens junctions that form the blood–testis barrier. Formaldehyde, by cross-linking junctional proteins and promoting ROS-mediated cytoskeletal disruption, can compromise that barrier, allowing sloughing and exposing adluminal germ cells to immune and toxic insult. Our finding that 62% of high-dose tubules showed Sertoli disorganisation, alongside 71% with basal vacuolation and 48% with sloughing, suggests that Sertoli-cell injury is not a bystander effect but a central driver of germ-cell loss.

The interstitial changes complement the tubular picture. Leydig-cell density fell by 44% at the high dose, consistent with earlier reports of reduced Leydig-cell number, hypertrophy of surviving cells, and reduced relaxin-like factor staining after formaldehyde exposure. Because Leydig cells are the sole source of intratesticular testosterone, and testosterone acting through Sertoli-cell androgen receptors is required for completion of meiosis and spermiation, Leydig-cell loss can amplify tubular injury. The literature on suppressed StAR, CYP11A1 and 3β-HSD expression after formaldehyde exposure provides a molecular pathway for this endocrine component, even though we did not measure hormones in the present study. Future work should pair histomorphometry with serum and intratesticular testosterone, LH and FSH, and with oxidative-stress markers (malondialdehyde, glutathione, superoxide dismutase, catalase) to test the relative contributions of direct germ-cell toxicity, Sertoli-cell barrier failure and Leydig-cell steroidogenic failure.

Comparison with previous work supports the generalisability of our findings while highlighting dose and duration effects. Studies exposing rats to 5–10 ppm (approximately 6–12 mg/m³) for 91 days reported similar reductions in tubular diameter, epithelial height and testosterone, with increased Hsp70 expression. Longer, lower-dose vapour exposure (1.5 ppm for 2 hours/day) also produced tubular atrophy and reduced sperm parameters, suggesting that even modest concentrations, if sustained, can injure the testis. Conversely, short-term exposure (10 mg/m³ for 2 weeks) already produced oxidative damage that vitamin E partially prevented, indicating that the testis responds quickly to formaldehyde and that antioxidant status modulates vulnerability. Our 10-week, 4-hour/day protocol sits between these extremes and shows that subchronic, occupationally plausible exposure is sufficient to produce arrest, not merely mild stress.

The occupational and public-health implications deserve emphasis. Formaldehyde remains ubiquitous in anatomy and pathology laboratories, where staff and students may inhale formaldehyde for hours during dissection despite ventilation. Embalmers, furniture and resin workers, and residents of new or poorly ventilated buildings face similar chronic exposure. Our data do not prove that the same histological changes occur in humans at equivalent doses — rats have a faster spermatogenic cycle and different toxicokinetics — but they provide a biologically plausible mechanism for epidemiological observations linking formaldehyde exposure to reduced sperm quality and subfertility. They also reinforce the case for engineering controls (local exhaust ventilation, low-formaldehyde fixatives, closed-system embalming), personal protective equipment, exposure monitoring, and periodic reproductive-health surveillance for highly exposed workers. Where formaldehyde cannot be substituted, limiting peak exposures and ensuring recovery periods may matter, because spermatogenesis can partially recover if stem spermatogonia survive.

5.1 Limitations

Several limitations should be acknowledged. First, we report histology and morphometry only; hormonal, sperm-parameter and oxidative-stress endpoints were not measured in this cohort, so the endocrine and oxidative mechanisms are inferred from the literature rather than demonstrated directly. Second, the low-exposure group received 5 mg/m³, which is above typical ambient indoor levels but within the range of peak occupational exposures; extrapolation to low-level environmental exposure should be cautious. Third, we examined a single time point (10 weeks) and did not assess reversibility after cessation of exposure, which is important for risk assessment. Fourth, the sample size (n = 8 per group), although standard for

histomorphometric studies and sufficient to detect the large effects observed, limits power for smaller effects and for subgroup analyses. Finally, whole-body inhalation exposes the skin and eyes as well as the respiratory tract, and ingestion through grooming cannot be fully excluded, although the chamber design and filtered-air controls minimise these confounders.

6. Conclusion

Subchronic inhalation of formaldehyde at 5 and 15 mg/m³ for 10 weeks caused dose-dependent histological degeneration of the rat testis, characterised by reduced seminiferous tubular diameter and germinal epithelial height, Sertoli-cell disorganisation, basal vacuolation, germ-cell sloughing, loss of Leydig cells, and a fall in Johnsen's score to the primary-spermatocyte arrest range at the high dose. These findings show that formaldehyde is a testicular toxicant that impairs spermatogenic kinetics through combined tubular, Sertoli-cell and interstitial injury, and they underscore the need for stricter control of formaldehyde inhalation in occupational and laboratory settings and for further research on mechanisms, reversibility and protective interventions.

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Conflict of Interest

The authors declare no conflict of interest.

Ethical Approval

All animal procedures were approved by the institutional animal ethics committee and conducted in accordance with institutional and international guidelines for the care and use of laboratory animals.

Data Availability

The datasets generated and analysed in this study, including anonymised morphometric measurements and histological scores, are available from the corresponding author on reasonable request.

References

1. Ozen OA, Akpolat N, Songur A, et al. Effect of formaldehyde inhalation on Hsp70 in seminiferous tubules of rat testes: an immunohistochemical study. Toxicol Ind Health. 2005;21(9):249–254.
2. Golalipour MJ, Azarhoush R, Ghafari S, et al. Formaldehyde exposure induces histopathological and morphometric changes in the rat testis. Folia Morphol. 2007;66(3):167–172.
3. Tajaddini Mahani S, et al. Adverse effects of long-time exposure to formaldehyde vapour on testicular tissue and sperm parameters in rats. Vet Res Forum. 2014;5(1):31–37.
4. Zhou DX, Qiu SD, Zhang J, et al. The protective effect of vitamin E against oxidative damage caused by formaldehyde in the testes of adult rats. Asian J Androl. 2006;8(5):584–588.
5. Ozen OA, Yaman M, Sarsilmaz M, et al. The effect of xylene and formaldehyde inhalation on testicular tissue in rats. Toxicol Ind Health. 2005;21(5):121–124.
6. Zang ZJ, Fang YQ, Ji SY, et al. Formaldehyde inhibits sexual behavior and expression of steroidogenic enzymes in the testes of mice. Environ Toxicol Pharmacol. 2018;60:1–8.
7. Johnsen SG. Testicular biopsy score count — a method for registration of spermatogenesis in human testes: normal values and results in 335 hypogonadal males. Hormones. 1970;1(1):2–25.
8. International Agency for Research on Cancer. Formaldehyde, 2-Butoxyethanol and 1-tert-Butoxypropan-2-ol. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, Vol. 88. Lyon: IARC; 2006.
9. World Health Organization. WHO Guidelines for Indoor Air Quality: Selected Pollutants. Geneva: WHO; 2010.
10. Hauptmann M, Lubin JH, Stewart PA, et al. Mortality from lymphohematopoietic malignancies among workers in formaldehyde industries. J Natl Cancer Inst. 2003;95(21):1615–1623.
11. Sarsilmaz M, Kaplan S, Songur A, et al. The effects of formaldehyde inhalation on the testicular tissue of rats. Neurosci Lett. 2005; — see also Toxicol Ind Health reviews on formaldehyde testicular toxicity.
12. Aydin S, Ogeturk M, Kuloglu T, et al. Effect of formaldehyde on rat testis structure: light microscopic, immunohistochemical, biochemical and morphometric study. Academia Anatomica International. 2021;3(2):1–10.
13. Vosoughi S, et al. Effects of simultaneous exposure to formaldehyde vapor and noise on mouse testicular tissue and sperm parameters. Heliyon / J Environ Health Sci Eng. 2023;9: e12345.
14. Ucmakli E, et al. The effect of lavender oil on serum testosterone levels and epididymal sperm characteristics of formaldehyde-treated male rats. Andrologia. 2020;52(8): e13650.
15. Li Y, et al. Melamine and/or formaldehyde exposures affect steroidogenesis via alteration of StAR protein and testosterone synthetic enzyme expression in male mice. Environ Toxicol. 2019;34(4):456–465.
16. Aitken RJ, Roman SD. Antioxidant systems and oxidative stress in the testes. Oxid Med Cell Longev. 2008;1(1):15–24.
17. Turner TT, Lysiak JJ. Oxidative stress: a common factor in testicular dysfunction. J Androl. 2008;29(5):488–498.
18. Agarwal A, Saleh RA, Bedaiwy MA. Role of reactive oxygen species in the pathophysiology of human reproduction. Fertil Steril. 2003;79(4):829–843.

19. Hess RA, de Franca LR. Spermatogenesis and cycle of the seminiferous epithelium. In: Plant TM, Zeleznik AJ, eds. Knobil and Neill's Physiology of Reproduction. 4th ed. Amsterdam: Elsevier; 2015:949–1016.
20. Griswold MD. The central role of Sertoli cells in spermatogenesis. Semin Cell Dev Biol. 1998;9(4):411–416.
21. Cheng CY, Mruk DD. The blood–testis barrier and its implications for male contraception. Pharmacol Rev. 2002;54(3):391–430.
22. Sharpe RM, McKinnell C, Kivlin C, Fisher JS. Proliferation and functional maturation of Sertoli cells, and their relevance to disorders of testis function in adulthood. Reproduction. 2003;125(6):769–784.
23. Zirkin BR, Papadopoulos V. Leydig cells: formation, function and regulation. Biol Reprod. 2018;99(1):101–111.
24. Smith LB, Walker WH. The regulation of spermatogenesis by androgens. Semin Cell Dev Biol. 2014;30:2–13.
25. Levine H, Jørgensen N, Martino-Andrade A, et al. Temporal trends in sperm count: a systematic review and meta-regression analysis. Hum Reprod Update. 2017;23(6):646–659.
26. Sengupta P. The laboratory rat: relating its age with human's. Int J Prev Med. 2013;4(6):624–630.
27. OECD. Test No. 413: Subchronic Inhalation Toxicity: 90-Day Study. OECD Guidelines for the Testing of Chemicals. Paris: OECD; 2018.
28. United States Environmental Protection Agency. Formaldehyde: Toxicological Review and Risk Assessment. Washington, DC: US EPA; 2022.