

Dose-Dependent Effects of a High-Sucrose Diet on Ovarian and Uterine Histomorphology and Hormonal Profile in Prepubertal Female Wistar Rats

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Abstract

High dietary sucrose intake has become a major public health concern because of its association with metabolic disorders and reproductive dysfunction. However, the dose-dependent effects of chronic sucrose consumption on female reproductive development during the prepubertal period remain poorly understood. This study investigated the effects of graded dietary sucrose concentrations on body weight, reproductive hormones, oxidative status, and the histomorphology of the ovaries and uterus in prepubertal female Wistar rats. Forty prepubertal female Wistar rats (3–4 weeks old; 50–60 g) were randomly assigned to four groups (n = 10/group). Group A received a standard diet and distilled water (control), while Groups B, C, and D received diets supplemented with 4%, 8%, and 16% sucrose, respectively, for 28 days. Body weight and feed intake were monitored throughout the experimental period. At study termination, blood samples were collected for hormonal and oxidative stress analyses, and ovarian and uterine tissues were harvested for histological evaluation using haematoxylin and eosin and Van Gieson staining. Data were analysed using one-way analysis of variance, with statistical significance set at $p < 0.05$. Sucrose supplementation produced dose-dependent increases in body weight and feed intake, with the greatest effects observed in the 8% and 16% sucrose groups. High-dose sucrose significantly increased progesterone concentrations while reducing luteinizing hormone, follicle-stimulating hormone, testosterone, and total antioxidant capacity compared with controls ($p < 0.05$). Histological examination demonstrated progressive ovarian follicular degeneration, granulosa cell disruption, stromal fibrosis, endometrial disorganization, glandular distortion, and increased collagen deposition, with the most pronounced alterations occurring in the 16% sucrose group. Chronic consumption of a high-sucrose diet during the prepubertal period induces dose-dependent endocrine imbalance, oxidative stress, and structural damage to the ovaries and uterus. These findings suggest that excessive dietary sucrose may adversely affect female reproductive development and could have long-term implications for reproductive health.

KEYWORDS: Diet reproductive hormones, Inflammation, Hormonal Imbalance, Dysmetabolism, Metabolism

1. Introduction

Diet plays a fundamental role in maintaining reproductive health, and recent global dietary shifts towards increased consumption of refined sugars have raised concerns regarding their impact on female reproductive function. Excessive sugar intake has been

strongly associated with obesity, insulin resistance, diabetes mellitus, and other metabolic disorders, all of which are recognized contributors to reproductive dysfunction [1]. The modern lifestyle, characterized by excessive caloric intake and physical inactivity, has fuelled an epidemic of metabolic diseases,

imposing a substantial burden on global public health [1]. Increasing evidence indicates that chronic consumption of sugar-rich diets promotes oxidative stress, inflammation, and endocrine dysregulation, thereby disrupting ovarian and uterine physiology and ultimately compromising female reproductive health [2,3].

Sucrose, the principal dietary disaccharide, is rapidly hydrolysed in the small intestine into glucose and fructose. While glucose is metabolized systemically under insulin regulation, fructose is predominantly metabolized in the liver. Excessive fructose intake stimulates *de novo* lipogenesis, promotes insulin resistance, increases the generation of reactive oxygen species, and activates pro-inflammatory cytokine pathways [4, 5]. These metabolic disturbances contribute to chronic oxidative stress and systemic inflammation, leading to widespread tissue injury and endocrine imbalance that adversely affect multiple organs, including the female reproductive system [4, 5].

The ovary and uterus are particularly susceptible to metabolic disturbances because their normal structure and function depend on tightly regulated endocrine and metabolic signalling. In the ovary, oxidative stress and insulin resistance impair folliculogenesis, steroidogenesis, granulosa cell proliferation, and oocyte maturation, resulting in follicular atresia, anovulation, and reduced fertility [7, 8]. Likewise, the uterus may exhibit endometrial disorganization, impaired receptivity, glandular distortion, and altered extracellular matrix remodelling, thereby reducing implantation potential and reproductive success [9–11]. Consequently, prolonged exposure to excessive dietary sucrose may adversely affect both the endocrine function and structural integrity of the female reproductive tract.

The prepubertal period represents a critical window of reproductive development during which the ovaries and uterus undergo rapid growth, differentiation, and maturation under endocrine regulation. Nutritional insults during this stage may permanently alter reproductive organ development and predispose individuals to reproductive disorders later in life [6]. Prepubertal female Wistar rats provide an established experimental model for reproductive toxicology because of their well-characterized reproductive physiology and developmental similarities to humans. Previous studies have shown that excessive dietary sugar intake during early life can impair ovarian follicular development, disrupt endocrine homeostasis, and induce morphological alterations resembling polycystic ovarian syndrome, including follicular atresia, anovulation, and reduced oocyte quality [7, 8]. Similarly, excessive sugar consumption has been associated with alterations in uterine morphology and function that may compromise implantation and fertility [9].

Although several studies have demonstrated the adverse metabolic effects of excessive sugar consumption, relatively few have comprehensively evaluated its dose-dependent effects on female reproductive development during the prepubertal period. Most previous investigations have focused either on metabolic

disturbances or reproductive hormonal changes, with limited attention to the integrated relationship between metabolic dysfunction, oxidative stress, endocrine alterations, and reproductive tissue architecture. Understanding these interactions is important because early-life nutritional insults may have lasting consequences for reproductive maturation and future fertility.

A 28-day exposure period was selected because it encompasses a substantial portion of the prepubertal developmental phase in Wistar rats and has been widely employed in sub-chronic nutritional studies to evaluate physiological and histopathological changes before sexual maturity. This duration permits the development of measurable metabolic, hormonal, and tissue alterations while minimizing confounding effects associated with adulthood. The graded dietary sucrose concentrations (4%, 8%, and 16%) were adopted from the experimental protocol of [5], which demonstrated that these concentrations produce progressive metabolic responses without causing excessive mortality or severe toxicity. The use of graded sucrose concentrations also enabled the assessment of dose-dependent responses and facilitated identification of the threshold at which metabolic disturbances begin to adversely affect reproductive tissues.

Despite increasing evidence linking high-sugar diets with metabolic disorders and reproductive dysfunction, information regarding the dose-dependent effects of chronic sucrose exposure during the prepubertal period on ovarian and uterine development remains limited. Addressing this knowledge gap is important because nutritional disturbances during this critical developmental window may exert long-term consequences on female reproductive health. Therefore, the present study investigated the dose-dependent effects of a high-sucrose diet on body weight, feed intake, blood glucose concentration, reproductive hormone profiles (progesterone, testosterone, luteinizing hormone, and follicle-stimulating hormone), oxidative stress biomarkers including malondialdehyde (MDA) and total antioxidant capacity (TAC), and the histomorphology of the ovaries and uterus in prepubertal female Wistar rats. We hypothesized that chronic consumption of graded concentrations of dietary sucrose would induce dose-dependent metabolic disturbances associated with oxidative stress, endocrine dysfunction, and progressive structural alterations in the female reproductive organs.

2. Materials and Methods

2.1 Experimental Animals

Healthy weaned female Wistar rats (3–4 weeks old; 50–60 g) were obtained from Empire Breeders, Osogbo, Osun State, Nigeria. The animals were housed in well-ventilated wooden cages (20 × 10 × 12 inches) under standard laboratory conditions at an ambient temperature of 25 ± 2.5°C, with a 12-h light/12-h dark photoperiod (lights on from 07:00 to 19:00 h). The rats were

allowed free access to standard rodent chow and clean drinking water *ad libitum* throughout the experimental period. All experimental procedures were approved by the Faculty of Basic Medical Sciences, Ladoke Akintola University of Technology (LAUTECH), Ogbomoso, Nigeria, and were conducted in accordance with the provisions for the care and use of laboratory animals prescribed by the European Council Directive (2010/63/EU) for the protection of animals used for scientific purposes.

2.2 Experimental Design

Forty (40) weaned female Wistar rats weighing 50–60 g and aged 3–4 weeks were assigned to four experimental groups (n = 10 per group). The animals were allocated to the groups without formal randomization or allocation concealment. Group A served as Control and were fed standard rodent chow and distilled water. Rats in group B, C and D were fed sucrose supplemented diet at 4, 8 and 16% (w/w) sucrose. Standard diet and sucrose diet were administered daily for 28 days. The experimental protocol and sucrose concentrations were adapted from the study by [5]. Experimental diets were prepared by thoroughly mixing 4 g, 8 g, and 16 g of sucrose with every 100 g of standard rodent chow to produce diets containing 4%, 8%, and 16% sucrose (w/w), respectively.

At the end of the 28-day treatment period, the animals were euthanized by cervical dislocation. Blood samples were collected for the determination of blood glucose, reproductive hormones, and oxidative stress biomarkers. The ovaries and uterus were carefully excised, fixed in 10% neutral buffered formalin, processed routinely for paraffin embedding, sectioned, and stained for histological evaluation.

2.3 Measurement of Body Weight and Feed Intake

Body weight and feed intake were monitored throughout the experimental period as previously described by [5]. Body weight was recorded weekly, whereas feed intake was measured daily using a calibrated electronic weighing balance.

2.4 Determination of Blood Glucose

Blood glucose concentrations were determined at the end of the treatment period using standard biochemical procedures.

2.5 Biochemical Analyses

2.5.1 Assessment of Lipid Peroxidation

Serum lipid peroxidation was assessed by determining malondialdehyde (MDA) concentration using the thiobarbituric acid reactive substances (TBARS) assay according to the method described by [12]. Malondialdehyde, a secondary product of lipid peroxidation, reacts with thiobarbituric acid (TBA) under acidic conditions at 95–100°C to form a pink chromogen (MDA–TBA complex). Following incubation in a boiling water bath for 60 minutes, the reaction mixture was cooled to room temperature and centrifuged to remove precipitated proteins. The absorbance of the supernatant was measured spectrophotometrically at 532 nm against a reagent blank. Malondialdehyde concentrations

were calculated from a standard curve prepared using 1,1,3,3-tetramethoxypropane and expressed as $\mu\text{mol/L}$.

2.5.2 Determination of Total Antioxidant Capacity

Total antioxidant capacity (TAC) was determined using the Trolox Equivalent Antioxidant Capacity (TEAC) assay according to the method described by [13]. This assay measures the ability of serum antioxidants to neutralize the pre-formed 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS^{•+}) radical cation, resulting in a reduction in colour intensity. After incubation with the ABTS working reagent, absorbance was measured spectrophotometrically at 734 nm. Antioxidant capacity was calculated from a Trolox standard curve and expressed as mmol Trolox equivalents/L.

2.5.3 Hormonal Assays

Serum concentrations of luteinizing hormone (LH), follicle-stimulating hormone (FSH), progesterone, and testosterone were quantified using commercially available rat enzyme-linked immunosorbent assay (ELISA) kits (Elabscience Biotechnology Co., Ltd., Wuhan, China) according to the manufacturer's instructions. Absorbance was measured at 450 nm using a microplate reader, and hormone concentrations were determined from standard calibration curves.

2.6 Histological Evaluation

The ovaries and uterus were fixed in 10% neutral buffered formalin, processed routinely, embedded in paraffin wax, sectioned at 5 μm , and stained with haematoxylin and eosin (H&E) for assessment of general tissue architecture. Van Gieson stain was employed to evaluate collagen deposition and connective tissue distribution within the reproductive organs.

2.7 Photomicrography

Histological sections were examined using an Olympus binocular light microscope, and representative photomicrographs were captured with a Canon PowerShot A2500 digital camera attached to the microscope. Histopathological evaluation was performed by a pathologist who was blinded to the treatment groups. Tissue sections were coded before microscopic examination to minimize observer bias.

2.8 Statistical Analysis

Data were analysed using Chris Rorden's ANOVA for Windows (Version 0.98). Results are presented as mean $\pm$ standard error of the mean (SEM). Before inferential analysis, data were assessed for normality and homogeneity of variance using the Shapiro–Wilk and Levene's tests, respectively.

Differences among the four experimental groups were analysed using one-way analysis of variance (ANOVA). When a significant overall effect was detected, Tukey's Honest Significant Difference (HSD) post hoc test was performed for pairwise comparisons between the control and each sucrose-treated group, as well as among the treatment groups (4% vs. 8%, 4% vs. 16%, and 8% vs. 16%). Statistical significance was accepted at $p < 0.05$.

3. Result

3.1 Effect of High-Sucrose Diet on Body Weight

The effects of graded dietary sucrose on body weight are presented in **Figure 1**. Rats fed diets containing 8% and 16% sucrose exhibited significantly greater body weight gain than the control group ($P < 0.05$). Although body weight increased slightly in the 4% sucrose group, this increase did not reach statistical significance.

3.2 Effect of High-Sucrose Diet on Feed Intake

As shown in **Figure 2**, feed intake increased progressively with increasing dietary sucrose concentration. Rats receiving the 16% sucrose diet consumed significantly more feed than the control group ($P < 0.05$), whereas feed intake in the 4% and 8% sucrose groups was not significantly different from controls.

3.3 Effect of High-Sucrose Diet on Reproductive Hormones

The effects of graded sucrose administration on reproductive hormone concentrations are presented in Tables 1 and 2. Progesterone concentrations decreased significantly in rats receiving the 4% sucrose diet, whereas animals fed the 16% sucrose diet demonstrated a marked increase compared with controls ($P < 0.05$). In contrast, testosterone concentrations declined significantly in all sucrose-treated groups, with the greatest reduction observed in the 16% sucrose group.

Luteinizing hormone (LH) exhibited a biphasic response, increasing significantly in the 8% sucrose group before declining in the 16% sucrose group. Follicle-stimulating hormone (FSH) increased following administration of 4% sucrose but decreased significantly in rats receiving 8% and 16% sucrose, indicating progressive disruption of pituitary–gonadal axis regulation with increasing dietary sucrose concentration.

3.4 Effect of High-Sucrose Diet on Oxidative Stress Markers

The effects of dietary sucrose on oxidative stress biomarkers are summarized in **Table 3**. Total antioxidant capacity (TAC) decreased following sucrose administration, with the greatest reduction observed in the 16% sucrose group, indicating impaired antioxidant defence. Malondialdehyde (MDA) concentrations differed significantly between the sucrose-treated groups and controls ($P < 0.05$), demonstrating that chronic dietary sucrose altered systemic oxidative status.

3.5 Effect of High-Sucrose Diet on Blood Glucose

Blood glucose concentrations are shown in **Figure 3**. No significant differences were observed between sucrose-treated groups and controls following the 28-day intervention ($P > 0.05$), indicating that graded dietary sucrose did not significantly alter fasting blood glucose during the study period.

3.6 Effect of High-Sucrose Diet on Uterine Histomorphology

Representative photomicrographs of haematoxylin and eosin (H&E)- and Verhoef's Van Gieson-stained uterine sections are presented in **Figures 4 and 5**, respectively. Uterine sections from the control group exhibited normal histological architecture characterized by an intact endometrium with numerous tortuous

uterine glands, a highly vascularized stroma, and well-organized myometrial smooth muscle. Verhoef's Van Gieson staining demonstrated normal connective tissue organization with minimal collagen deposition. In contrast, rats receiving 8% and 16% sucrose exhibited progressive histopathological alterations, including degeneration of endometrial glands, stromal disorganization, cellular degeneration, and increased collagen deposition, indicative of uterine fibrosis. These changes were most pronounced in the 16% sucrose-treated group.

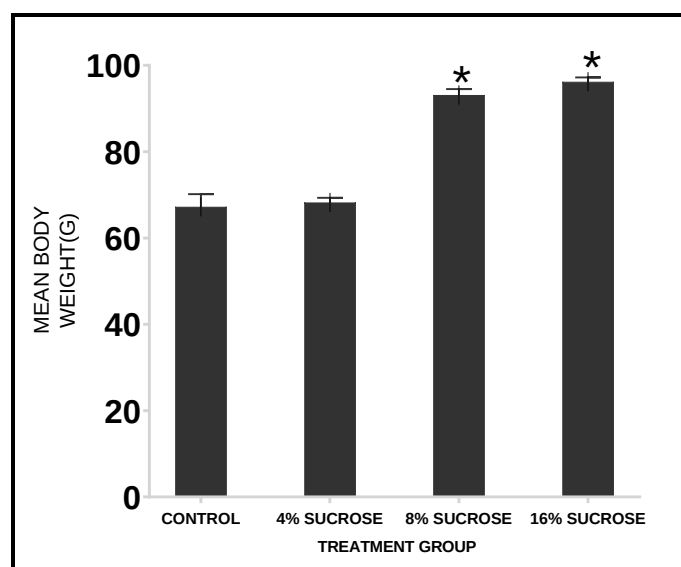


Figure 1. Effect of graded dietary sucrose on body weight. Values are expressed as Mean $\pm$ SEM ($P < 0.05$ versus control; $n = 10$ per group).

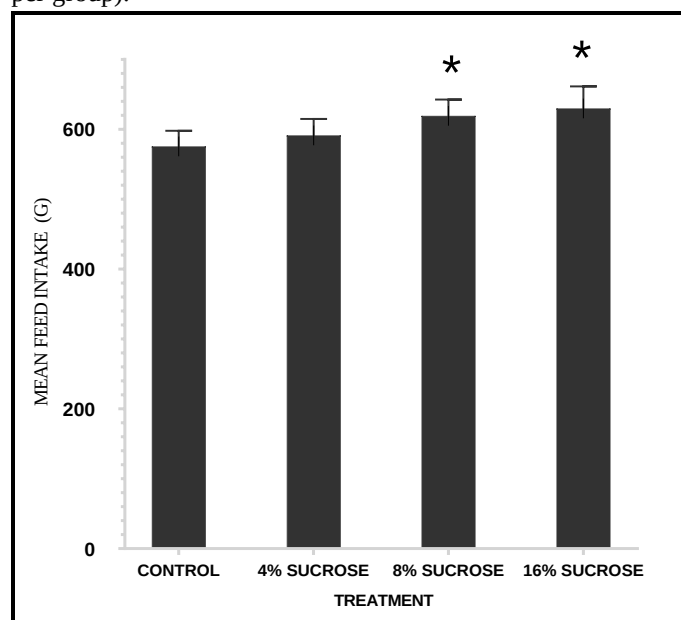


Figure 2. Effect of graded dietary sucrose on feed intake. Values are expressed as Mean $\pm$ SEM ($P < 0.05$ versus control; $n = 10$ per group).

Table 1. Effect of Graded Dietary Sucrose on Serum Progesterone and Testosterone Levels in Prepubertal Female Wistar Rats

Group	Progesterone (pg/mL)	Testosterone (ng/mL)
Control	0.97 ± 0.02	0.67 ± 0.05
4% Sucrose	0.24 ± 0.08*	0.34 ± 0.01*
8% Sucrose	0.99 ± 0.23	0.23 ± 0.01*
16% Sucrose	3.64 ± 0.32*	0.11 0.06*

Values are presented as mean ± SEM (n = 10 per group). *Significantly different from the control group (P < 0.05).

Table 2. Effect of Graded Dietary Sucrose on Serum Luteinizing Hormone and Follicle-Stimulating Hormone Levels in Prepubertal Female Wistar Rats

Group	Luteinizing Hormone (mIU/mL)	Follicle-Stimulating Hormone (mIU/mL)
Control	2.81±0.28	24.36±4.91
4% Sucrose	3.71±0.38	52.95±22.34
8% Sucrose	6.81±2.04*	4.37±1.07*
16% Sucrose	2.30±0.57*	3.19±0.77*

Values presented as S mean ± SEM (n = 10 per group). *Significantly different from the control group (P < 0.05).

3.7 Effect of High-Sucrose Diet on Ovarian Histomorphology

Representative H&E- and Verhoef’s Van Gieson-stained ovarian sections are shown in **Figures 6 and 7**, respectively. Control ovaries displayed normal histoarchitecture with well-preserved primordial, primary, secondary, and Graafian follicles, intact granulosa and theca cell layers, and normal corpora lutea. Progressive ovarian degeneration was observed following sucrose administration. Rats receiving 8% and 16% sucrose exhibited disruption of follicular architecture, degeneration of granulosa cells, poorly defined oocytes, increased follicular atresia, and stromal fibrosis. Verhoef’s Van Gieson staining further demonstrated progressive collagen accumulation within the ovarian stroma and around developing follicles, particularly in the 16% sucrose-treated group.

Table 3. Effect of Graded Dietary Sucrose on Oxidative Stress Biomarkers in Prepubertal Female Wistar Rats

Group	Malondialdehyde (MDA) (µmol/L)	Total Antioxidant Capacity (TAC) (mmol Trolox equivalents/L)
Control	4.42 ± 0.07	18.45 ± 2.83
4% Sucrose	2.17 ± 0.40*	15.66 ± 0.44
8% Sucrose	0.82 ± 0.01*	18.69 ± 1.57
16% Sucrose	1.48 ± 0.31*	12.10 ± 2.27

Values are presented as mean ± SEM (n = 10 per group). *Significantly different from the control group (P < 0.05)

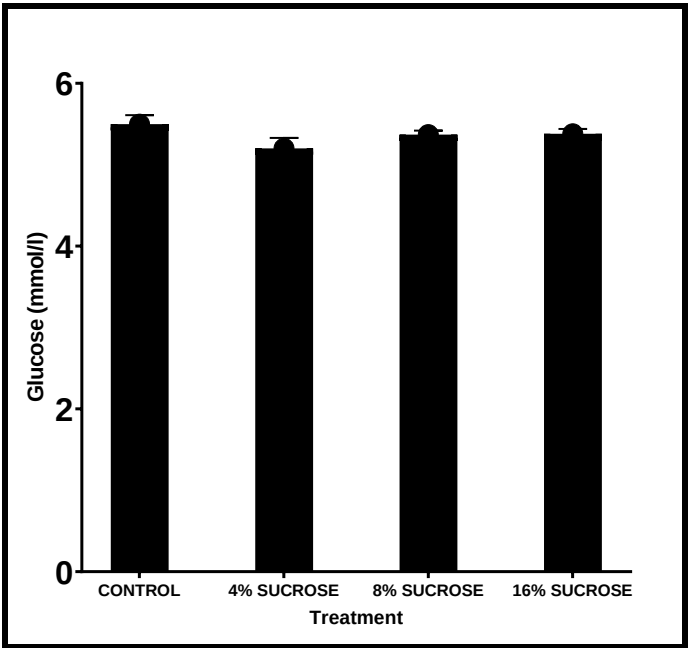


Figure 3. Effect of graded dietary sucrose on blood glucose concentration. Values are expressed as Mean ± SEM (n = 10 per group).

3.8 Quantitative Histomorphometric Analysis of the Uterus (Haematoxylin and Eosin)

Quantitative histomorphometric analysis demonstrated dose-dependent deterioration of uterine architecture following sucrose administration (**Table 4**). Compared with controls, rats receiving 8% and 16% sucrose exhibited significant reductions (P < 0.05) in endometrial gland density, gland diameter, gland lumen area, epithelial height, endometrial thickness, and percentage glandular area. Conversely, stromal cell density and percentage

stromal area increased significantly, indicating progressive stromal expansion and disruption of normal uterine architecture. Mild, non-significant changes were observed in the 4% sucrose group

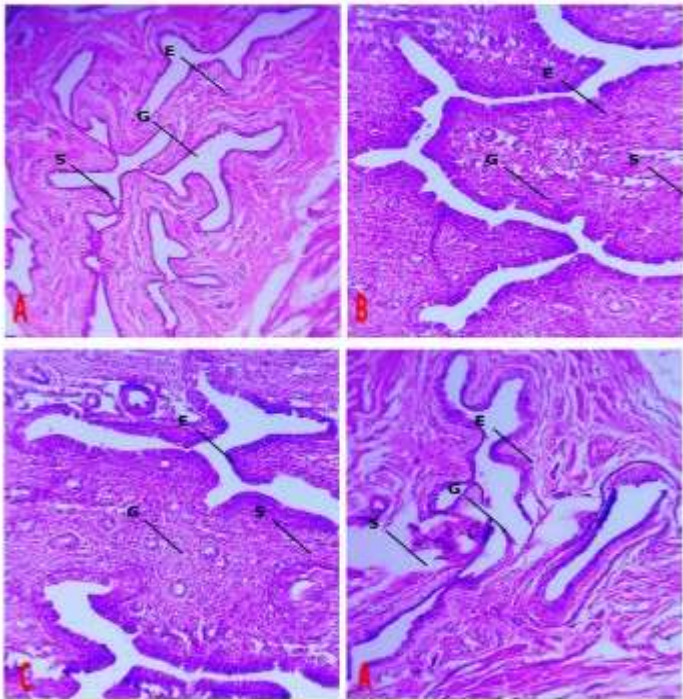


Figure 4: Representative photomicrograph of uterus Stained by Haematoxylin & eosin X100 Mag. It reveals uterine structures Endometrium (E), Uterine gland lumen (G), Stroma (S).

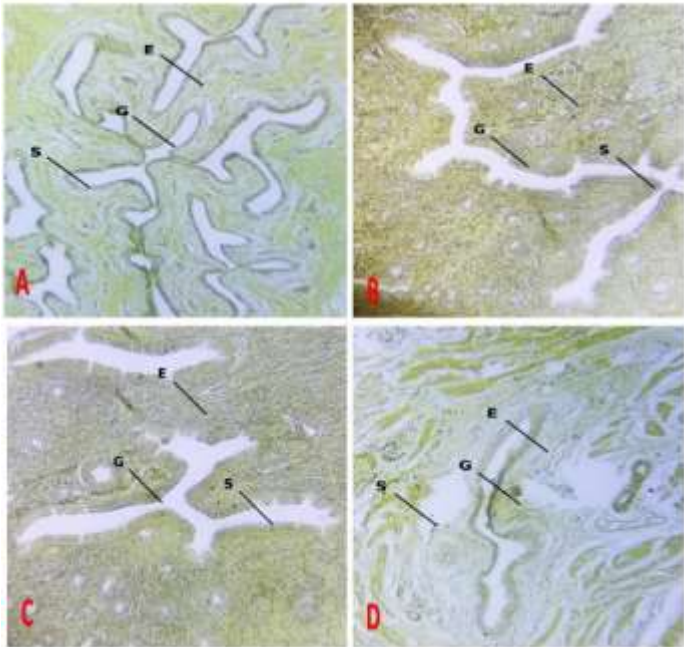


Figure 5: Representative photomicrograph of uterus Stained by Verhoef's Van Gieson X100 Mag. The stained slides revealed uterine structures Endometrial gland (E), Stroma (S), Uterine gland lumen (G).

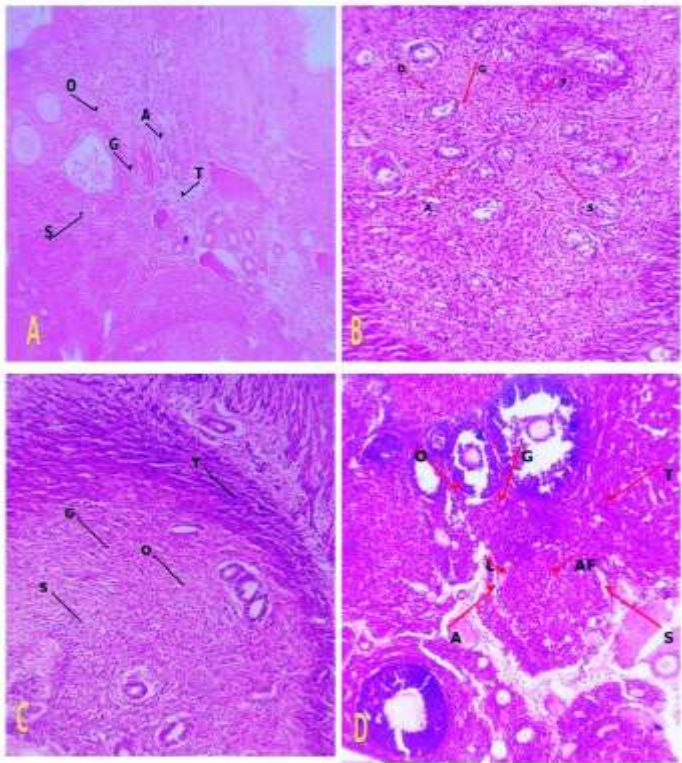


Figure 6: Representative photomicrograph of ovary Stained by Hematoxylin & eosin X100 Mag. The ovarian structures are labelled Oocyte(O), Granulosa cells (G), Theca cells (T), Stroma (S); Antrum (A); Atretic follicle (AF).

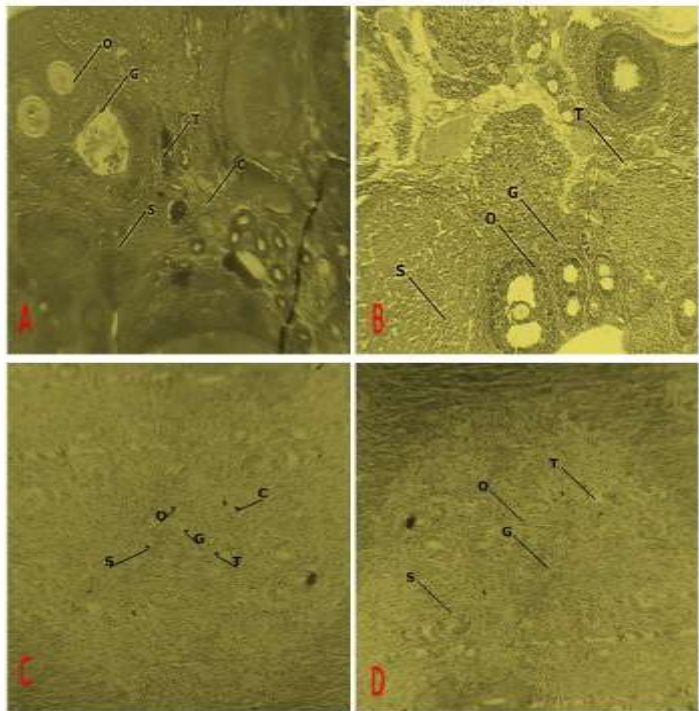


Figure 7: Representative photomicrograph ovary Stained by Van Gieson X100 Mag. The ovarian structures are labelled; Oocyte (O), Granulosa cells (G), Theca cells (T), Stroma (S).

3.9 Quantitative Histomorphometric Analysis of the Uterus (Van Gieson)

Histomorphometric assessment of Van Gieson-stained uterine sections revealed progressive connective tissue remodelling with increasing sucrose concentration (Table 5). Rats fed 8% and 16% sucrose demonstrated significantly higher ($P < 0.05$) collagen area fraction, stromal collagen density, stromal thickness, blood vessel wall thickness, and fibrosis scores than controls. Conversely, gland perimeter and gland circularity decreased significantly, indicating progressive collagen deposition, stromal remodelling, and uterine fibrosis.

Table 4. Effect of Graded Dietary Sucrose on Uterine Histomorphometric Parameters (Haematoxylin and Eosin Stain)

Histomorphometric Parameter	Control	4% Sucrose	8% Sucrose	16% Sucrose
Endometrial gland density (glands/mm ²)	26.80 ± 1.12	23.60 ± 1.08	19.20 ± 0.95*	14.80 ± 0.84*
Mean glandular diameter (µm)	62.54 ± 2.31	57.12 ± 2.06	49.43 ± 1.84*	41.26 ± 1.65*
Gland lumen area (µm ²)	1726.4 ± 81.5	1542.8 ± 74.6	1288.6 ± 69.2*	1024.8 ± 61.4*
Luminal epithelial height (µm)	17.86 ± 0.62	16.42 ± 0.58	14.28 ± 0.46*	11.96 ± 0.41*
Stromal cell density (×10 ² cells/mm ²)	2.18 ± 0.10	2.46 ± 0.11	2.88 ± 0.12*	3.42 ± 0.14*
Endometrial thickness (µm)	318.4 ± 9.8	301.2 ± 10.1	276.6 ± 8.9*	238.5 ± 7.6*
Glandular area (%)	31.62 ± 1.24	28.41 ± 1.15	23.54 ± 0.98*	17.82 ± 0.91*
Stromal area (%)	68.38 ± 1.24	71.59 ± 1.15	76.46 ± 0.98*	82.18 ± 0.91*

Values are presented as mean ± SEM (n = 10 per group). *Significantly different from the control group ($P < 0.05$) as determined by one-way ANOVA followed by Tukey's post hoc test.

3.10 Quantitative Histomorphometric Analysis of the Ovary (Haematoxylin and Eosin)

Quantitative ovarian histomorphometry demonstrated marked dose-dependent impairment of follicular development (Table 6). Compared with controls, rats receiving 8% and 16% sucrose exhibited significant reductions ($P < 0.05$) in primordial, primary, secondary, and Graafian follicle densities, granulosa cell layer thickness, theca cell layer thickness, follicular diameter, and corpus luteum density. In contrast, ovarian stromal cell density

and follicular atresia increased significantly, indicating progressive follicular degeneration and impaired ovarian function. Only mild structural alterations were observed in the 4% sucrose group.

Table 5. Effect of Graded Dietary Sucrose on Uterine Connective Tissue Histomorphometric Parameters (Verhoeff's Van Gieson Stain)

Histomorphometric Parameter	Control	4% Sucrose	8% Sucrose	16% Sucrose
Collagen area fraction (%)	12.8 ± 0.8	16.4 ± 0.9	23.7 ± 1.1*	31.6 ± 1.4*
Stromal collagen density (%)	14.2 ± 0.7	18.1 ± 0.8	25.8 ± 1.2*	34.5 ± 1.5*
Endometrial stromal thickness (µm)	96.4 ± 3.8	103.2 ± 4.2	116.8 ± 4.6*	132.6 ± 5.3*
Endometrial gland perimeter (µm)	286.5 ± 11.6	274.2 ± 10.8	251.8 ± 9.6*	224.6 ± 8.9*
Endometrial gland circularity	0.92 ± 0.02	0.88 ± 0.02	0.81 ± 0.03*	0.73 ± 0.03*
Blood vessel wall thickness (µm)	12.6 ± 0.5	14.1 ± 0.6	16.8 ± 0.7*	19.4 ± 0.8*
Fibrosis score (0–3)	0.3 ± 0.1	0.8 ± 0.2	1.8 ± 0.2*	2.7 ± 0.2*

Values are presented as mean ± SEM (n = 10 per group). *Significantly different from the control group ($P < 0.05$) as determined by one-way ANOVA followed by Tukey's post hoc test. Histomorphometric measurements were calibrated using a scale conversion factor of 9 µm/pixel.

3.11 Quantitative Histomorphometric Analysis of the Ovary (Verhoff's Van Gieson)

Verhoff's Van Gieson staining demonstrated marked dose-dependent connective tissue remodelling within the ovary (Table 7). Rats treated with 8% and 16% sucrose exhibited significant increases ($P < 0.05$) in ovarian collagen area fraction, stromal collagen density, stromal thickness, collagen surrounding follicles and blood vessels, interstitial stromal area, and fibrosis scores compared with controls. These findings indicate progressive extracellular matrix accumulation, stromal fibrosis, and structural deterioration of ovarian tissue following chronic high-sucrose consumption.

Discussion

This study investigated the dose-dependent effects of chronic dietary sucrose consumption on reproductive endocrine function, oxidative stress, and uterine and ovarian histomorphology in

prepubertal female Wistar rats. The findings demonstrate that prolonged exposure to increasing concentrations of dietary sucrose induces progressive metabolic and reproductive alterations characterized by hormonal dysregulation, impairment of antioxidant defence, and marked structural remodelling of both the uterus and ovaries. These changes were accompanied by quantitative histomorphometric evidence of extracellular matrix expansion and fibrosis, indicating that excessive sucrose consumption during the prepubertal period adversely affects the development and integrity of female reproductive organs. Overall, the severity of these alterations increased with sucrose concentration, suggesting a clear dose-dependent relationship between dietary sucrose exposure and reproductive toxicity. These findings corroborate previous reports demonstrating that excessive dietary sugar intake disrupts reproductive physiology through metabolic dysfunction, oxidative stress, and chronic low-grade inflammation [14–16].

Table 6. Effect of Graded Dietary Sucrose on Ovarian Histomorphometric Parameters (Haematoxylin and Eosin Stain)

Histomorphometric Parameter	Group A (Control)	Group B (4% Sucrose)	Group C (8% Sucrose)	Group D (16% Sucrose)
Primordial follicle density (follicles/mm ²)	18.40 ± 0.84	16.80 ± 0.76	14.20 ± 0.65*	10.60 ± 0.58*
Primary follicle density (follicles/mm ²)	12.60 ± 0.71	11.20 ± 0.62	8.80 ± 0.51*	6.40 ± 0.43*
Secondary follicle density (follicles/mm ²)	8.20 ± 0.46	7.00 ± 0.42	5.20 ± 0.35*	3.60 ± 0.28*
Graafian follicle density (follicles/mm ²)	3.80 ± 0.26	3.20 ± 0.24	2.20 ± 0.18*	1.20 ± 0.12*
Atretic follicle density (follicles/mm ²)	1.20 ± 0.10	2.40 ± 0.18	4.80 ± 0.31*	7.20 ± 0.42*
Granulosa cell layer thickness (µm)	46.8 ± 1.9	42.6 ± 1.7	36.4 ± 1.4*	29.8 ± 1.2*
Theca cell layer thickness (µm)	21.6 ± 0.9	20.2 ± 0.8	18.0 ± 0.7*	15.8 ± 0.6*
Mean follicular diameter (µm)	218.6 ± 8.4	202.8 ± 7.9	178.4 ± 6.6*	151.2 ± 5.8*
Ovarian stromal cell density (×10 ² cells/mm ²)	2.34 ± 0.12	2.58 ± 0.13	3.04 ± 0.14*	3.66 ± 0.16*
Corpus luteum density (corpora lutea/mm ²)	2.40 ± 0.18	2.00 ± 0.16	1.40 ± 0.12*	0.80 ± 0.08*

Values are presented as mean ± SEM (n = 10 per group). *Significantly different from the control group (*P* < 0.05) as determined by one-way ANOVA followed by Tukey's post hoc test. Histomorphometric measurements were calibrated using a scale conversion factor of 9 µm/pixel.

Table 7. Effect of Graded Dietary Sucrose on Ovarian Connective Tissue Histomorphometric Parameters (Verhoef's Van Gieson Stain)

Histomorphometric Parameter	Group A (Control)	Group B (4% Sucrose)	Group C (8% Sucrose)	Group D (16% Sucrose)
Ovarian collagen area fraction (%)	10.8 ± 0.6	14.6 ± 0.8	21.8 ± 1.0*	30.4 ± 1.3*
Stromal collagen density (%)	12.4 ± 0.7	16.8 ± 0.9	24.6 ± 1.1*	33.8 ± 1.5*
Ovarian stromal thickness (µm)	104.6 ± 4.2	112.8 ± 4.6	126.4 ± 5.1*	145.8 ± 5.8*
Perifollicular collagen deposition (%)	8.6 ± 0.5	11.4 ± 0.6	17.8 ± 0.8*	24.2 ± 1.1*
Perivascular collagen deposition (%)	11.2 ± 0.6	14.8 ± 0.7	20.6 ± 0.9*	28.4 ± 1.2*
Interstitial stromal area (%)	28.6 ± 1.2	31.8 ± 1.4	36.8 ± 1.6*	42.6 ± 1.8*
Fibrosis score (0–3)	0.2 ± 0.1	0.8 ± 0.1	1.7 ± 0.2*	2.8 ± 0.2*

Values are presented as mean ± SEM (n = 10 per group). *Significantly different from the control group (*P* < 0.05) as determined by one-way analysis of variance (ANOVA) followed by Tukey's post hoc test. Histomorphometric measurements were calibrated using a scale conversion factor of 9 µm/pixel.

The observed changes in body weight further support the metabolic consequences of chronic sucrose consumption. Rats maintained on the control diet exhibited the expected physiological pattern of weight gain characteristic of normal growth during the prepubertal period, whereas animals receiving sucrose-enriched diets demonstrated progressive alterations in body weight. Although administration of 4% sucrose produced only a modest increase in body weight, the 8% sucrose diet resulted in significantly greater weight gain, indicating enhanced adiposity associated with increased dietary sugar intake. Interestingly, animals receiving the 16% sucrose diet displayed a less consistent pattern of weight gain despite continued sucrose exposure. This observation may reflect the onset of metabolic dysregulation accompanying prolonged excessive sugar

consumption. Chronic intake of sucrose, particularly its fructose component, promotes hepatic de novo lipogenesis, increases triglyceride synthesis, and stimulates adipocyte hypertrophy while simultaneously reducing peripheral insulin sensitivity. The resulting hyperinsulinaemia favours lipid storage, suppresses lipolysis, and ultimately contributes to progressive adiposity and weight gain (Petersen & Shulman, 2023; Samuel & Shulman, 2024). However, as metabolic dysfunction advances, alterations in substrate utilization, mitochondrial efficiency, and energy expenditure may attenuate further weight gain despite continued caloric excess. These findings are consistent with previous reports demonstrating that chronic sucrose consumption promotes obesity and metabolic dysfunction in experimental animals [14–16].

Feed intake also increased progressively with dietary sucrose concentration, particularly among animals receiving the 8% and 16% sucrose diets. The stable food consumption observed in the control group reflects normal hypothalamic regulation of appetite and energy homeostasis, whereas the hyperphagic response observed in sucrose-treated animals suggests disruption of these regulatory mechanisms. High dietary sugar intake is known to influence central reward pathways through dopaminergic neurotransmission, thereby enhancing the hedonic value of palatable foods and promoting increased consumption beyond normal physiological requirements [16,17]. Furthermore, chronic sucrose exposure has been associated with impaired insulin and leptin signalling, both of which play critical roles in appetite regulation and satiety. Reduced hypothalamic sensitivity to these hormones may diminish satiety responses, thereby perpetuating excessive food intake and further exacerbating metabolic dysfunction. Consequently, the increased feed intake observed in the present study likely reflects the combined effects of increased caloric density, altered reward signalling, and endocrine dysregulation. Similar findings have been reported in previous studies demonstrating that chronic consumption of sugar-rich diets disrupts normal feeding behaviour and contributes to obesity-related metabolic abnormalities [15–17].

The endocrine alterations observed in this study indicate that chronic sucrose consumption disrupts the normal regulation of the hypothalamic–pituitary–ovarian (HPO) axis. Gonadotropin secretion exhibited a biphasic response to increasing sucrose concentrations, with moderate sucrose exposure producing transient stimulation followed by suppression at the highest concentration. Serum luteinizing hormone increased in animals receiving 8% sucrose but declined following administration of the 16% sucrose diet, suggesting that prolonged metabolic stress may initially stimulate pituitary activity before ultimately impairing gonadotropic function. Follicle-stimulating hormone demonstrated a similar pattern, with a modest increase following low-dose sucrose administration but significant reductions at higher concentrations. Such changes are consistent with

progressive disruption of pituitary regulation resulting from chronic metabolic stress, oxidative injury, and altered hypothalamic feedback mechanisms.

Steroid hormone production was also markedly affected. The reduction in progesterone observed following administration of 4% sucrose may represent an early manifestation of impaired ovarian steroidogenesis resulting from oxidative damage to granulosa and luteal cells. In contrast, the marked elevation of progesterone observed in the 16% sucrose group likely reflects abnormal persistence of luteinized cells, altered steroid metabolism, or compensatory activation of steroidogenic pathways in response to extensive ovarian injury rather than normal luteal function. Progressive reductions in circulating testosterone across the sucrose-treated groups further indicate impairment of ovarian steroidogenic capacity. Collectively, these endocrine abnormalities provide a plausible mechanistic explanation for the extensive follicular degeneration, impaired folliculogenesis, and uterine glandular alterations observed in the present study and are consistent with previous reports linking excessive dietary sugar intake with reproductive endocrine dysfunction [14,15].

Oxidative stress appears to represent a central mechanism underlying the reproductive toxicity induced by chronic sucrose consumption. Total antioxidant capacity declined significantly following administration of sucrose, particularly in animals receiving the highest dietary concentration, indicating progressive depletion of endogenous antioxidant defenses. The reduction in antioxidant capacity suggests that antioxidant reserves were increasingly consumed in an attempt to neutralize excessive reactive oxygen species generated during sucrose metabolism. Fructose metabolism is known to accelerate mitochondrial reactive oxygen species production through enhanced hepatic lipogenesis, impaired mitochondrial oxidative phosphorylation, activation of inflammatory signalling pathways, and the development of insulin resistance [18,19]. Sustained oxidative stress can subsequently impair cellular proteins, lipids, nucleic acids, and mitochondrial function, thereby disrupting normal reproductive physiology and tissue homeostasis.

Interestingly, serum malondialdehyde concentrations declined rather than increased following sucrose administration. Although seemingly paradoxical, this finding does not necessarily contradict the evidence of oxidative stress. Malondialdehyde reflects lipid peroxidation and depends not only on reactive oxygen species generation but also on membrane lipid composition, antioxidant activity, and the stage of oxidative injury. During the early adaptive phase of oxidative stress, endogenous antioxidant systems may preferentially suppress lipid peroxidation despite overall depletion of antioxidant reserves. Furthermore, chronic metabolic stress may alter membrane phospholipid composition or increase the metabolic clearance of lipid peroxidation products, resulting in lower circulating

malondialdehyde concentrations despite persistent oxidative imbalance [18,19]. Consequently, interpretation of malondialdehyde should be considered alongside total antioxidant capacity rather than as an isolated indicator of oxidative status.

Despite the metabolic and hormonal disturbances observed, fasting blood glucose concentrations remained relatively unchanged across the experimental groups. Maintenance of normoglycaemia during the study period likely reflects compensatory hyperinsulinaemia, whereby increased pancreatic β -cell insulin secretion offsets the developing peripheral insulin resistance induced by chronic sucrose consumption. Such compensation is characteristic of the early stages of metabolic dysfunction and frequently precedes impaired glucose tolerance and overt diabetes mellitus [20,21]. The relatively short duration of dietary exposure may also have been insufficient to overwhelm pancreatic β -cell reserve. In addition, prepubertal animals possess greater metabolic flexibility and enhanced β -cell functional capacity than mature animals, enabling them to preserve glucose homeostasis despite substantial metabolic stress [18,19]. These observations suggest that reproductive endocrine dysfunction and tissue injury may develop before overt disturbances in fasting glucose become clinically apparent.

Histological evaluation of the uterus demonstrated progressive structural deterioration with increasing dietary sucrose concentration, indicating that chronic sucrose exposure adversely affects uterine development during the prepubertal period. The uterine architecture of the control animals was preserved, exhibiting a well-organized endometrium with intact luminal epithelium, abundant endometrial glands, highly cellular stroma, and a normal myometrial arrangement. These findings are consistent with the normal histological organization of the mammalian uterus and reflect an appropriate hormonal environment for endometrial growth and differentiation [22]. In contrast, sucrose-treated animals exhibited a dose-dependent progression from mild epithelial alterations in the 4% sucrose group to extensive glandular degeneration, stromal disorganization, epithelial disruption, and architectural distortion in the 16% sucrose group. The progressive nature of these lesions suggests that chronic exposure to excessive dietary sucrose progressively compromises uterine integrity rather than producing an acute toxic effect.

The observed uterine lesions are likely multifactorial and reflect the combined effects of endocrine dysregulation, oxidative stress, and chronic metabolic inflammation. Endometrial growth and cyclical remodelling are tightly regulated by ovarian steroid hormones, particularly oestrogen and progesterone. Consequently, disruption of the hypothalamic–pituitary–ovarian axis, together with impaired ovarian steroidogenesis, would be expected to alter epithelial proliferation, glandular differentiation, and stromal organization. Furthermore, excessive generation of

reactive oxygen species may directly damage endometrial epithelial and stromal cells through lipid peroxidation, mitochondrial dysfunction, and activation of pro-inflammatory signalling pathways, thereby accelerating tissue degeneration.

Chronic inflammatory activation also stimulates fibroblast proliferation and extracellular matrix synthesis, providing a mechanistic link between oxidative stress and the progressive fibrotic changes observed in the present study. One of the most important qualitative findings was the progressive degeneration of the luminal epithelium. The luminal epithelium constitutes the initial site of embryo attachment during implantation, and preservation of its structural integrity is essential for successful establishment of pregnancy. Degeneration of this epithelial layer may therefore impair uterine receptivity and reduce implantation potential [22]. Similarly, the marked distortion and reduction of endometrial glands observed in the higher sucrose-treated groups suggest impaired uterine secretory activity. Endometrial glands produce histotroph containing growth factors, cytokines, nutrients, and adhesion molecules required for blastocyst survival and implantation. Structural disruption of these glands may therefore compromise endometrial function long before overt infertility becomes clinically apparent.

Verhoef's Van Gieson staining provided additional evidence that connective tissue remodelling represents a major component of sucrose-induced uterine injury. Progressive collagen deposition was observed across the treatment groups, with mild accumulation following administration of 4% sucrose, moderate fibrosis in the 8% group, and extensive stromal fibrosis in animals receiving the 16% sucrose diet. Excessive collagen accumulation within the uterine stroma is a characteristic feature of chronic tissue injury and reflects persistent activation of fibroblasts and excessive extracellular matrix synthesis. Such fibrotic remodelling reduces tissue compliance, interferes with normal endometrial remodelling, and may ultimately impair implantation and successful maintenance of pregnancy [22]. The severity of fibrosis observed in the present study strongly suggests that chronic sucrose consumption promotes pathological connective tissue remodelling rather than simple inflammatory infiltration.

The ovaries exhibited a similar dose-dependent pattern of structural deterioration. Ovarian sections from control animals displayed normal cortical architecture characterized by follicles at various stages of development, intact granulosa and theca cell layers, healthy oocytes, and well-defined corpora lutea, consistent with normal ovarian maturation in prepubertal animals. Administration of 4% sucrose produced only subtle histological alterations with preservation of overall follicular architecture, suggesting that relatively low levels of dietary sucrose induce only early adaptive responses. However, increasing dietary sucrose concentration resulted in progressively more severe ovarian injury. Animals receiving 8% sucrose demonstrated disruption of granulosa cell organization, irregular antral cavities,

and increased follicular atresia, whereas those receiving the 16% sucrose diet exhibited widespread follicular degeneration, oocyte disruption, extensive follicular atresia, and marked distortion of normal ovarian architecture.

These morphological alterations are consistent with impairment of folliculogenesis secondary to endocrine and metabolic disturbances. Normal follicular development requires coordinated interactions between granulosa cells, theca cells, gonadotropins, and locally produced growth factors. Oxidative stress disrupts these interactions by inducing mitochondrial dysfunction, DNA damage, apoptosis, and impaired steroidogenic enzyme activity within granulosa cells. The marked reduction in circulating gonadotropins and alterations in ovarian steroid hormones observed in the present study provide additional evidence that disruption of endocrine regulation contributed substantially to the observed histological changes. Consequently, the progressive follicular degeneration is unlikely to represent direct tissue toxicity alone but rather reflects the combined effects of oxidative injury, hormonal imbalance, and chronic metabolic stress acting on the developing ovary. Verhoef's Van Gieson staining further demonstrated progressive connective tissue remodelling within the ovarian stroma. Mild collagen accumulation was evident following administration of 4% sucrose, whereas moderate fibrosis developed in the 8% sucrose group and extensive collagen deposition characterized the ovaries of animals receiving the highest sucrose concentration. Progressive ovarian fibrosis has important functional implications because excessive extracellular matrix accumulation alters the biochemical and mechanical properties of the ovarian microenvironment. Increased stromal stiffness may mechanically restrict follicular expansion and ovulation while simultaneously impairing communication between stromal cells, granulosa cells, and developing oocytes. Furthermore, collagen accumulation surrounding ovarian blood vessels may compromise tissue perfusion, reducing oxygen and nutrient delivery to growing follicles and thereby accelerating follicular degeneration. These mechanisms collectively provide a plausible explanation for the marked impairment of follicular development observed following chronic sucrose administration. Together, the qualitative histological findings indicate that chronic dietary sucrose induces progressive structural injury in both the uterus and ovary through interconnected mechanisms involving endocrine dysfunction, oxidative stress, chronic inflammation, and extracellular matrix remodelling. The parallel deterioration observed in both reproductive organs suggests that excessive sucrose consumption affects the female reproductive system as an integrated endocrine unit rather than producing isolated organ-specific effects. This integrated response provides a strong biological basis for the quantitative histomorphometric alterations discussed in the subsequent sections and supports the hypothesis that chronic

high-sucrose intake during the prepubertal period may have lasting consequences for female reproductive health.

The quantitative histomorphometric findings provide objective evidence that chronic sucrose consumption profoundly alters uterine microarchitecture in a dose-dependent manner. Unlike the qualitative histological observations, which primarily demonstrate structural alterations, histomorphometric analysis permits precise quantification of tissue remodelling and therefore strengthens the evidence for sucrose-induced uterine toxicity. The progressive reductions in endometrial gland density, glandular diameter, gland lumen area, epithelial height, endometrial thickness, and glandular area observed in the present study indicate that excessive dietary sucrose impairs normal endometrial development and glandular maturation. These quantitative findings complement the histological observations and demonstrate that structural deterioration of the uterus extends beyond isolated cellular injury to involve significant architectural remodelling.

Endometrial glands are indispensable for normal reproductive function because they synthesize and secrete histotroph, a complex mixture of nutrients, cytokines, growth factors, lipids, and adhesion molecules that supports embryo survival before placental establishment [22]. The significant reduction in gland density and glandular dimensions observed in the 8% and 16% sucrose groups therefore suggests impaired secretory capacity of the uterus. Such alterations may compromise endometrial receptivity by reducing the availability of glandular secretions required for blastocyst implantation and early embryonic development. Similar reductions in glandular development have been reported in experimental models of oxidative stress, endocrine disruption, and metabolic syndrome, where impaired glandular differentiation has been associated with reduced fertility and implantation failure [23,24].

The reduction in luminal epithelial height observed in the present study provides additional evidence of impaired endometrial maturation. The luminal epithelium constitutes the first point of contact between the blastocyst and the maternal endometrium, and its structural integrity is essential for embryo adhesion, implantation, and subsequent placentation [22]. Maintenance of epithelial morphology depends largely on coordinated estrogen and progesterone signalling. Consequently, the hormonal disturbances observed in the sucrose-treated animals likely contributed to the progressive epithelial thinning. Oxidative stress may further exacerbate epithelial injury through mitochondrial dysfunction, disruption of cytoskeletal organization, and activation of apoptotic pathways, thereby limiting the regenerative capacity of the endometrial lining. The observed reduction in epithelial height therefore reflects both endocrine dysfunction and direct oxidative injury.

Endometrial thickness also declined progressively with increasing sucrose concentration. Normal endometrial growth

depends upon tightly regulated cycles of epithelial proliferation, stromal expansion, angiogenesis, and extracellular matrix turnover under the influence of ovarian steroid hormones. Reduced endometrial thickness therefore suggests impaired cellular proliferation and diminished responsiveness to hormonal stimulation. Such alterations have important reproductive implications because inadequate endometrial development is associated with reduced implantation rates, impaired decidualization, and adverse pregnancy outcomes [23]. The progressive reduction observed in this study therefore supports the hypothesis that chronic sucrose consumption compromises uterine preparedness for successful reproduction.

Conversely, stromal cell density and stromal area increased significantly following administration of higher sucrose concentrations. Expansion of the stromal compartment is characteristic of chronic tissue injury and frequently reflects fibroblast activation, inflammatory cell infiltration, and excessive extracellular matrix deposition. Persistent oxidative stress stimulates numerous profibrotic signalling pathways, including transforming growth factor- β (TGF- β), connective tissue growth factor (CTGF), and platelet-derived growth factor (PDGF), all of which promote fibroblast proliferation and collagen synthesis [24,25]. Consequently, the increase in stromal cellularity observed in the present study likely represents an early stage of pathological tissue remodelling preceding overt fibrosis. The simultaneous reduction in glandular area and expansion of stromal tissue further indicates a shift in tissue composition from functional glandular elements toward fibrotic stromal components, a hallmark of chronic uterine injury.

The Verhoef's Van Gieson histomorphometric findings provide compelling quantitative evidence that connective tissue remodelling constitutes a major pathological consequence of chronic sucrose exposure. Collagen area fraction and stromal collagen density increased progressively across the treatment groups, demonstrating dose-dependent accumulation of extracellular matrix within the uterine stroma. Excessive collagen deposition is widely recognized as a defining feature of fibrosis and reflects an imbalance between extracellular matrix synthesis and degradation [24]. Reactive oxygen species generated during chronic metabolic stress activate fibroblasts and stimulate collagen synthesis through TGF- β -mediated signalling pathways while simultaneously inhibiting matrix metalloproteinases responsible for collagen degradation. The resulting extracellular matrix accumulation progressively replaces normal functional tissue with fibrotic connective tissue, thereby compromising uterine elasticity and function.

The significant increase in endometrial stromal thickness further supports the occurrence of pathological stromal expansion. Increased stromal thickness may result from a combination of fibroblast proliferation, inflammatory cell infiltration, oedema, and excessive deposition of collagen and other extracellular

matrix proteins. These changes reduce tissue compliance and may interfere with the cyclical structural remodelling required for successful implantation and pregnancy maintenance. Similar stromal expansion has been reported in experimental models of obesity, diabetes, and endocrine disruption, conditions characterized by chronic oxidative stress and low-grade inflammation [24–26]. Thus, the present findings reinforce the concept that excessive dietary sucrose can reproduce pathological uterine changes typically associated with metabolic disease.

Additional evidence of progressive glandular distortion was provided by the reductions in endometrial gland perimeter and gland circularity. These morphometric indices objectively quantify glandular shape and structural integrity. The significant reduction in gland perimeter indicates shrinkage of glandular structures, whereas the decline in gland circularity reflects increasing irregularity and distortion of gland morphology. Such alterations are consistent with degeneration of glandular epithelium and compression of glandular lumina by expanding stromal connective tissue. Consequently, the morphometric evidence demonstrates that chronic sucrose exposure disrupts not only gland number but also gland architecture, thereby further compromising secretory function.

Blood vessel wall thickness also increased significantly with increasing dietary sucrose concentration. Vascular remodelling is a common consequence of chronic inflammation and oxidative stress and frequently accompanies progressive tissue fibrosis. Thickening of the vascular wall may impair uterine perfusion by reducing vascular compliance and altering local blood flow. Inadequate perfusion may subsequently exacerbate tissue hypoxia, promote additional oxidative stress, and stimulate further fibrogenesis through activation of hypoxia-inducible signalling pathways. This positive feedback mechanism may partly explain the progressive nature of the structural alterations observed in the present study.

The progressive increase in fibrosis score provides an integrated histopathological measure of connective tissue remodelling and confirms that collagen accumulation translated into overt fibrotic change. The marked increase observed in animals receiving the 16% sucrose diet indicates advanced uterine fibrosis and supports the morphometric findings of increased collagen deposition, stromal expansion, and glandular degeneration. Fibrosis is generally regarded as the final common pathway of chronic tissue injury and frequently results in irreversible impairment of organ function. Within the uterus, excessive fibrosis reduces tissue plasticity, interferes with normal endometrial remodelling, and may impair embryo implantation and placental development [25,26].

Collectively, the uterine histomorphometric findings demonstrate that chronic dietary sucrose induces coordinated structural remodelling involving glandular regression, epithelial atrophy, stromal expansion, vascular remodelling, and progressive

collagen accumulation. These alterations provide quantitative evidence that excessive sucrose consumption during the prepubertal period disrupts normal uterine maturation through interconnected mechanisms involving endocrine dysfunction, oxidative stress, chronic inflammation, and extracellular matrix remodelling. The close agreement between the qualitative histological observations and quantitative morphometric data considerably strengthens the evidence that chronic high-sucrose intake adversely affects uterine structure and may ultimately compromise reproductive competence.

The quantitative histomorphometric evaluation of the ovary provides robust evidence that chronic dietary sucrose consumption progressively impairs ovarian development during the prepubertal period. While the qualitative histological findings demonstrated progressive follicular degeneration and stromal disruption, quantitative morphometry objectively confirmed significant reductions in primordial, primary, secondary, and Graafian follicle densities with increasing sucrose concentration. These findings indicate that chronic sucrose exposure adversely affects multiple stages of folliculogenesis, suggesting that the toxic effects are not restricted to mature follicles but extend to the ovarian reserve itself. Since the primordial follicle pool determines the reproductive lifespan of females, reductions in primordial follicle density may have long-term consequences for fertility by accelerating depletion of the ovarian reserve [27].

Normal follicular development depends upon coordinated endocrine regulation involving follicle-stimulating hormone, luteinizing hormone, locally produced growth factors, and an intact ovarian microenvironment. The progressive reductions in follicle density observed in the present study are therefore consistent with the hormonal disturbances demonstrated earlier. Suppression of gonadotropin secretion and disruption of ovarian steroidogenesis are likely to impair recruitment, maturation, and survival of developing follicles. In addition, oxidative stress may directly damage granulosa cells through mitochondrial dysfunction, DNA fragmentation, and activation of intrinsic apoptotic pathways. Because granulosa cells provide metabolic and structural support to developing oocytes, their degeneration inevitably compromises follicular growth and accelerates follicular atresia. These observations are consistent with previous studies demonstrating that metabolic stress and excessive reactive oxygen species impair follicular development and reduce ovarian reserve [27,28].

The marked increase in atretic follicle density further supports the conclusion that excessive sucrose consumption promotes premature follicular degeneration. Follicular atresia represents the physiological mechanism through which non-dominant follicles are eliminated; however, excessive atresia reflects pathological disruption of ovarian homeostasis. Reactive oxygen species have been shown to activate caspase-dependent apoptotic pathways within granulosa cells, resulting in follicular collapse

and degeneration. Furthermore, insulin resistance and chronic inflammation alter the intraovarian cytokine environment, thereby promoting apoptosis while suppressing cellular proliferation and tissue repair. Consequently, the increased follicular atresia observed in the present study likely reflects the combined effects of endocrine dysfunction, oxidative injury, and inflammatory activation. Progressive loss of viable follicles may ultimately reduce reproductive capacity and impair future fertility.

Significant reductions in granulosa cell layer thickness and theca cell layer thickness provide additional evidence of impaired follicular maturation. Granulosa cells play essential roles in estrogen synthesis, follicular nourishment, and bidirectional communication with the oocyte, whereas theca cells provide androgen precursors required for estrogen biosynthesis. Damage to either cellular compartment disrupts steroidogenesis and compromises follicular development. Oxidative stress has been shown to inhibit aromatase activity, impair mitochondrial cholesterol transport, and suppress steroidogenic enzyme expression, thereby reducing ovarian hormone production [28]. The observed thinning of both granulosa and theca cell layers therefore reflects not only structural degeneration but also functional impairment of ovarian endocrine activity. These findings correspond closely with the alterations in circulating reproductive hormones demonstrated in the present study and reinforce the central role of oxidative stress in mediating sucrose-induced ovarian toxicity.

The reduction in follicular diameter observed across the treatment groups provides further evidence of arrested follicular growth. Follicular enlargement requires coordinated granulosa cell proliferation, antral fluid accumulation, extracellular matrix remodelling, and adequate vascular support. Chronic oxidative stress interferes with these processes by disrupting cellular metabolism, reducing angiogenic signalling, and impairing granulosa cell proliferation. Consequently, follicles exposed to persistent metabolic stress fail to achieve normal preovulatory maturation, resulting in smaller follicular diameters and increased susceptibility to atresia. The concurrent reduction in corpus luteum density further suggests impaired ovulation or defective luteinization. Because the corpus luteum is responsible for progesterone production following ovulation, reductions in corpus luteum formation may contribute to the endocrine abnormalities observed in the present study and indicate compromised ovarian function beyond follicular development alone.

Another important observation was the significant increase in ovarian stromal cell density following administration of higher sucrose concentrations. Expansion of the ovarian stroma is characteristic of chronic ovarian injury and frequently accompanies fibrosis and extracellular matrix remodelling. Activated stromal fibroblasts produce large quantities of collagen

and other extracellular matrix proteins in response to oxidative stress and inflammatory cytokines, thereby progressively replacing functional ovarian tissue with fibrotic connective tissue. Excessive stromal proliferation has also been reported in several reproductive disorders associated with metabolic dysfunction, including obesity and polycystic ovary syndrome, where increased stromal volume contributes to altered ovarian function [28,29]. The present findings therefore suggest that chronic sucrose consumption induces pathological stromal remodelling that may further impair follicular development and endocrine activity.

The Verhoef's Van Gieson histomorphometric findings provide compelling quantitative evidence that chronic dietary sucrose induces progressive ovarian fibrosis. Significant increases in ovarian collagen area fraction, stromal collagen density, perifollicular collagen deposition, perivascular collagen deposition, stromal thickness, interstitial stromal area, and fibrosis score collectively demonstrate extensive extracellular matrix accumulation within the ovarian cortex. These changes indicate that oxidative injury extends beyond follicular degeneration to involve widespread connective tissue remodelling. Fibrosis develops when collagen synthesis exceeds degradation, resulting in progressive replacement of functional parenchyma with dense extracellular matrix. Reactive oxygen species, together with inflammatory mediators such as transforming growth factor- β , stimulate fibroblast activation while suppressing matrix metalloproteinases responsible for collagen degradation, thereby promoting irreversible tissue remodelling [24–29].

Perifollicular collagen accumulation is particularly significant because the mechanical properties of the extracellular matrix play an essential role in follicular growth and ovulation. Excessive collagen surrounding developing follicles may restrict follicular expansion, impair rupture of the follicular wall during ovulation, and alter communication between granulosa cells and the surrounding stroma. Similarly, increased collagen surrounding ovarian blood vessels may reduce vascular compliance and tissue perfusion, limiting oxygen and nutrient delivery to developing follicles while exacerbating oxidative stress. The significant increase in stromal thickness and interstitial stromal area observed in this study further indicates progressive replacement of functional ovarian tissue with fibrotic connective tissue, thereby reducing the capacity of the ovary to support normal folliculogenesis.

The progressive increase in fibrosis score represents the cumulative consequence of these connective tissue changes and provides strong evidence that chronic sucrose exposure promotes advanced ovarian fibrosis. Importantly, fibrosis is generally regarded as a largely irreversible pathological process once established. Therefore, exposure to excessive dietary sucrose during the critical prepubertal period may produce persistent

structural alterations that extend into reproductive maturity. These findings have important developmental implications because early-life nutritional insults are increasingly recognized as determinants of adult reproductive health through developmental programming mechanisms. Chronic metabolic disturbances during this critical developmental window may permanently alter ovarian architecture, endocrine function, and reproductive potential.

Collectively, the qualitative histological observations, quantitative histomorphometric analyses, hormonal alterations, and oxidative stress findings demonstrate a highly coordinated pattern of reproductive toxicity induced by chronic dietary sucrose consumption. Rather than representing isolated pathological events, these alterations appear to constitute an integrated pathological cascade initiated by metabolic dysregulation. Excessive sucrose intake promotes oxidative stress and inflammatory activation, resulting in disruption of hypothalamic–pituitary–ovarian axis function, impairment of steroidogenesis, degeneration of uterine and ovarian cellular architecture, progressive extracellular matrix accumulation, and ultimately fibrosis of both reproductive organs. The remarkable agreement between the biochemical, endocrine, histological, and histomorphometric findings considerably strengthens the internal consistency of the study and supports the conclusion that oxidative stress-mediated endocrine dysfunction represents a central mechanism underlying sucrose-induced reproductive toxicity.

The present study also provides important translational insights into the potential consequences of excessive dietary sugar consumption during adolescence and early reproductive development. Although extrapolation from animal models to humans should be undertaken cautiously, the findings raise concerns regarding the increasing consumption of sugar-rich diets among children and adolescents worldwide. Persistent exposure to high-sucrose diets during critical developmental periods may predispose females to reproductive disorders characterized by impaired folliculogenesis, altered uterine receptivity, ovarian fibrosis, and reduced fertility later in life. Consequently, reducing excessive dietary sugar intake during early life may represent an important preventive strategy for preserving female reproductive health.

Overall, this study demonstrates that chronic graded dietary sucrose consumption induces dose-dependent reproductive toxicity in prepubertal female Wistar rats through interconnected mechanisms involving endocrine dysregulation, oxidative stress, impaired folliculogenesis, pathological uterine remodelling, and progressive fibrosis of both the uterus and ovary. These findings provide comprehensive experimental evidence that excessive dietary sucrose adversely affects the structural and functional development of female reproductive organs and underscore the importance of limiting excessive sugar consumption during

critical periods of reproductive maturation. Further studies investigating the molecular pathways underlying these changes, particularly oxidative stress signalling, inflammatory mediators, apoptotic pathways, and extracellular matrix regulatory mechanisms, will enhance understanding of the mechanisms responsible for diet-induced reproductive dysfunction and may identify potential therapeutic targets for preventing or reversing these pathological alterations.

Conclusion

Chronic consumption of graded dietary sucrose during the prepubertal period induced dose-dependent reproductive toxicity in female Wistar rats. Excessive sucrose intake disrupted reproductive hormone homeostasis, impaired antioxidant status, and produced progressive histopathological and histomorphometric alterations in the ovaries and uterus, including impaired folliculogenesis, endometrial gland degeneration, increased collagen deposition, and fibrosis. These findings suggest that oxidative stress-mediated endocrine dysfunction and extracellular matrix remodelling are key mechanisms underlying sucrose-induced reproductive injury. Overall, the study highlights the potential adverse effects of excessive dietary sugar consumption on female reproductive development and underscores the need for further mechanistic studies to identify strategies for preventing diet-induced reproductive dysfunction.

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Availability of data and materials

Data generated during and analysed during the course of this study are available from the corresponding author on request.

Competing interests

All authors of this paper declare that there is no conflict of interest related to the content of this manuscript.

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