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# Impact of chronic monosodium glutamate exposure on female reproductive health in an animal model

*Dampak paparan kronis monosodium glutamat terhadap kesehatan reproduksi perempuan pada model hewan*

Fathunikmah[1*], Siti Mona Amelia Lestari[2], Ani Laila[3], Zahira Abyan Putri Wahyudhi[4]

[1] Health Polytechnic of the Ministry of Health, Riau, Pekanbaru, Indonesia.
E-mail: fathunikmah@pkr.ac.id
[2] Faculty of Medicine, University of Muhammadiyah Prof. Dr. Hamka, Jakarta, Indonesia.
E-mail: sitimona@uhamka.ac.id
[3] Health Polytechnic of the Ministry of Health, Riau, Pekanbaru. Indonesia.
E-mail: ani@pkr.ac.id
[4] Faculty of Teacher Training and Education, University of Muhammadiyah Prof. Dr. Hamka, Jakarta, Indonesia.
E-mail: ontaskzahirabyan@gmail.com

***Correspondence Author:**
Health Polytechnic of the Ministry of Health Riau. 103 Melur Street, Harjosari, Sukajadi, Pekanbaru, Riau 28156, Indonesia.
E-mail: fathunikmah@pkr.ac.id

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## Abstract

Monosodium glutamate (MSG) is a widely used food additive; however, its chronic effects on female reproductive health remain unclear. Previous studies have mainly focused on neurotoxic and metabolic outcomes, leaving a gap in understanding its impact on ovarian function. This study investigated the effects of chronic MSG exposure on ovarian structure and follicular development in female mice. An experimental post-test-only control group design was used at the Biomedical Laboratory, Poltekkes Kemenkes Riau, Indonesia, from August to October 2024. Twenty-four female Swiss mice (Mus musculus), aged 8–10 weeks and weighing 25–30 g, were randomly divided into four groups (n = 6 per group). The control group received standard feed, while the treatment groups were administered MSG orally at low (0,25 g/kg body weight/day), medium (1 g/kg body weight/day), and high (4 g/kg body weight/day) doses for eight weeks. Ovarian tissues were examined using histopathology and flow cytometry. Data were analyzed using one-way analysis of variance (ANOVA), post-hoc tests, and correlation analysis. The medium- and high-dose groups showed significant reductions in primary (12,3 ± 2,1; 8,7 ± 1,9) and secondary follicles (7,8 ± 1,5; 4,9 ± 1,2), accompanied by tissue degeneration and germ cell apoptosis. A strong negative correlation was observed between MSG dose and mature follicle count ($r = -0,72$; $p < 0,01$). In conclusion, these findings demonstrate dose-dependent ovarian impairment, underscoring the need for dietary risk evaluation and increased public awareness regarding excessive MSG consumption.ChE is not strong enough to assess changes in body composition clinically.

**Keywords:** Apoptosis, monosodium glutamate, oogenesis, ovarian health, reproductive toxicology

## Abstrak

Monosodium glutamate (MSG) merupakan bahan tambahan pangan yang banyak digunakan, namun efek paparan kronisnya terhadap kesehatan reproduksi perempuan masih belum sepenuhnya dipahami. Penelitian sebelumnya lebih banyak menyoroti dampak neurotoksik dan metabolik, sehingga terdapat kesenjangan pengetahuan mengenai pengaruhnya terhadap fungsi ovarium. Penelitian bertujuan menganalisis efek paparan MSG kronis terhadap struktur ovarium dan perkembangan folikel pada mencit betina. Desain penelitian menggunakan post-test-only control group yang dilaksanakan di Laboratorium Biomedik Poltekkes Kemenkes Riau, Indonesia, pada Agustus–Oktober 2024. Sebanyak 24 ekor mencit Swiss betina (Mus musculus), berusia 8–10 minggu dengan berat 25–30 g, dibagi secara acak ke dalam empat kelompok (n = 6). Kelompok kontrol mendapat pakan standar, sedangkan kelompok perlakuan diberi MSG per oral dengan dosis rendah (0,25 g/kg BB/hari), sedang (1 g/kg BB/hari), dan tinggi (4 g/kg BB/hari) selama delapan minggu. Jaringan ovarium diperiksa menggunakan histopatologi dan sitometri. Analisis menggunakan uji one-way ANOVA, uji post-hoc, dan korelasi. Hasil menunjukkan penurunan signifikan jumlah folikel primer (12,3 ± 2,1; 8,7 ± 1,9) dan sekunder (7,8 ± 1,5; 4,9 ± 1,2) pada kelompok dosis sedang dan tinggi, disertai degenerasi

jaringan dan apoptosis sel germinal. Korelasi negatif kuat ditemukan antara dosis MSG dan jumlah folikel matang ($r = -0,72$; $p < 0,01$). Kesimpulan, paparan MSG kronis berdampak merusak ovarium secara dosis-tergantung, sehingga perlu evaluasi risiko konsumsi dan peningkatan kesadaran masyarakat.

**Kata Kunci:** Apoptosis, kesehatan ovarium, monosodium glutamat, oogenesis, toksikologi reproduksi

## Introduction

Monosodium glutamate (MSG) or sodium glutamate is a salt compound of glutamic acid that is naturally present in various protein sources; however, it is widely used as a food additive to enhance umami flavor (Wijayasekara & Wansapala, 2021). Glutamic acid is a non-essential amino acid that plays an important role in protein metabolism, acts as a neurotransmitter precursor, and contributes to energy production in cells, making it nutritionally relevant at moderate intake (Osawa, 2022). Since its first commercial production in the early 20th century, MSG has become one of the most widely consumed additives worldwide, particularly in Asian countries, including Indonesia. The popularity of MSG in culinary practices is growing not only because of its ability to enrich flavors but also because of its high availability, affordability, and ease of use in a wide variety of foods (Banerjee et al., 2021). However, behind these culinary benefits, there are growing scientific concerns about the long-term impact of MSG consumption, especially if consumed in excess and continuously in the daily diet (Kayode et al., 2023).

In the context of food regulation, MSG has been recognized as safe for consumption in limited quantities. The Joint FAO/WHO Expert Committee on Food Additives (JECFA) has granted it Generally Recognized as Safe (GRAS) status, with an acceptable daily intake (ADI) of up to 30 mg/kg body weight/day (Kayode et al., 2023). However, this provision is based on classical toxicology data, which assumes that consumption remains within reasonable limits. In reality, modern society's increasing reliance on processed and instant foods, often without awareness or control over the additive content, has led to a chronic increase in MSG exposure (Reid & Price, 2023). This condition opens the possibility of subclinical toxic effects that may not be detected through short-term monitoring or standard metabolic biomarkers.

Most previous studies on the impact of MSG have focused on its neurotoxic effects, oxidative stress, and metabolic disorders, such as metabolic syndrome, type 2 diabetes, and hepatic dysfunction (Kesherwani et al., 2024). However, research on the chronic effects of MSG on female reproductive health remains limited. In particular, studies comprehensively addressing ovarian morphology, oogenesis, and the mechanisms of follicular apoptosis in relation to long-term MSG exposure are scarce.

This research gap highlights the need for further investigation of the reproductive toxicity of MSG. Moreover, mechanisms previously reported in other organs, such as oxidative stress, mitochondrial dysfunction, and metabolic dysregulation, are also highly relevant to the physiology of the ovary. Ovarian function, particularly oogenesis and folliculogenesis, relies heavily on stable hormonal regulation, energy metabolism, and a redox balance. Therefore, the toxic mechanisms observed in neural and metabolic tissues may compromise ovarian health, potentially leading to reduced follicle reserves, impaired fertility, and long-term endocrine disturbances.

Women's reproductive health, particularly ovarian function, is highly sensitive to exposure to exogenous chemicals because oogenesis and folliculogenesis are highly dependent on hormonal balance, enzymatic activity, and stable micro-and macronutrient status. Disruption of these processes can lead to infertility, decreased ovarian reserves, and long-term endocrine disorders (Al-Otaibi et al., 2022).

In nutritional toxicology studies, MSG should be considered a form of nutrient or non-nutrient exposure in the daily diet that may exert cumulative effects on target organs, including the ovaries (Nazam et al., 2023). Beyond their role as gamete producers, the ovaries function as endocrine glands that regulate the systemic hormonal balance, particularly estrogen and progesterone production (Al-Suhaimi et al., 2022). Chronic MSG exposure has been associated with oxidative stress and potential disruption of the hypothalamic–pituitary–ovarian (HPO) axis signaling, which can impair hormonal regulation. Such disturbances may alter

oogenesis and folliculogenesis, leading to structural and functional changes in the ovaries. Consequently, disruptions in ovarian morphology and physiology can lead to menstrual irregularities, reduced fertility, and adverse pregnancy outcomes (Das et al., 2023). These effects extend beyond individuals, contributing to broader public health concerns, including declining birth rates, an increased risk of idiopathic infertility, and greater reliance on assisted reproductive technologies.

However, studies examining the effects of MSG in the context of female reproduction are limited, particularly those that use an integrative approach combining histopathology, cytometry, and nutritional risk analysis methods. Animal models, such as mice (Mus musculus), are still a relevant translational approach for evaluating the systemic effects of food additives on vital organs (Rinninella et al., 2021). Animal-based experimental research allows for the direct observation of tissue structure, cellular dynamics, and potential dysfunctions that may not be directly measurable in human epidemiological studies (Sarmento et al., 2025).

Therefore, this study aimed to assess the effects of chronic MSG exposure on ovarian morphology and oogenesis dynamics in female mice, using them as an animal model. In contrast to previous studies that have primarily focused on MSG's neurotoxic and metabolic effects of MSG, this study specifically addresses the underexplored gap concerning its long-term impact on female reproductive health at both the structural and cellular levels. By combining histological and cytometric approaches, this study aimed to identify the structural and functional changes resulting from prolonged MSG consumption, which were interpreted within the framework of nutritional toxicology. The findings are expected to contribute to the scientific understanding of MSG's reproductive impact and provide a foundation for advocating safer additive consumption policies and strengthening community nutrition education, particularly among women of childbearing age, who are physiologically vulnerable to reproductive dysfunction resulting from uncontrolled dietary exposures.

# Methods

### Research Design

This study employed a laboratory experimental design with a post-test-only control group approach. The study was conducted at the Biomedical Laboratory, Poltekkes Kemenkes Riau, Pekanbaru, Indonesia, from August to October 2024. This design was chosen to directly observe structural and functional changes in ovarian morphology and oogenesis dynamics after chronic dietary exposure to Monosodium Glutamate (MSG). MSG dosages, low (0,25 g/kg body weight/day), medium (1 g/ body weight/day), and high (4 g/kg body weight/day), were selected based on previous toxicological studies to represent levels ranging from commonly consumed dietary amounts to potentially excessive exposures. This approach allowed for the assessment of both physiological tolerance and dose-dependent toxic effects.

### Subject and Procedure of Treatment

The study involved 24 Swiss female rats (Mus musculus), aged 8–10 weeks, with an average body weight of 25–30 g, obtained from an accredited biomedical laboratory. The rats were housed in individual cages under controlled room conditions with a temperature of 22–24°C and a 12-hour light-dark cycle, with ad libitum access to standard feed and drinking water.

All the test animals underwent a 7-day adaptation period before treatment. After adaptation, the rats were randomly divided into four groups of six animals. The sample size of 24 was determined considering previous toxicological studies using similar designs and was deemed adequate to detect medium-to-large effect sizes with sufficient statistical power (≥80%) for ANOVA analysis. The K0 group (control) received only standard feed without the addition of monosodium glutamate (MSG). The K1 group (low-dose) was administered MSG at 0,25 g/kg body weight/day, the K2 group (moderate-dose) received 1 g/kg body weight/day, and the K3 group (high-dose) was administered 4 g/kg body weight/day.

MSG was administered orally through daily gavage for eight weeks, following a chronic exposure protocol consistent with sub chronic toxicology principles. The selected doses were determined based on conversions from estimated human dietary overconsumption into rodent-equivalent doses using body surface area normalization, as well as prior studies that documented histological alterations in target organs under chronic MSG exposure.

### Histopathology and Cytometry Evaluation

At the end of the treatment, all the test animals were sacrificed using a terminal anesthesia

protocol. The ovaries were fixed in 10% formalin and processed using the paraffin-block method. Sections were prepared at a thickness of 5 μm and stained with hematoxylin and eosin (HE) for histopathological examination. The observed parameters included the general ovarian structure, number and morphology of follicles (primordial, primary, secondary, and Graafian), presence of corpus luteum, and signs of tissue degeneration or necrosis. Cytometric analysis was conducted using digital microscopy combined with semiquantitative image analysis software (ImageJ, NIH, USA). The software facilitated the measurement of follicle size, count, and distribution, as well as the quantification of apoptotic cells and structural changes in oogenesis with a higher precision. All observations were independently assessed by two blinded researchers to minimize interpretation bias and improve the reliability of the findings (Fineschi, 2022).

**Data Analysis**

Before conducting the analysis, data normality was assessed using the Shapiro–Wilk test, which was selected because of its high sensitivity and suitability for small to moderate sample sizes, making it appropriate for this study, which included 24 animals. The results showed that the data for variables X and Y were normally distributed ($p > 0,05$), whereas the data for variable Z were not ($p < 0,05$). For normally distributed variables, one-way analysis of variance (ANOVA) was applied to compare mean values among groups, followed by Tukey's HSD post-hoc test to detect specific pairwise differences (Zhuang et al., 2021). For non-normally distributed data, the Kruskal–Wallis test was employed as a non-parametric alternative to ANOVA, allowing comparison of median values across groups without assuming normality. When significant differences were found, the Mann–Whitney U test was used for pairwise comparisons. Statistical significance was set at $p < 0,05$. All analyses were performed using SPSS software (version 26).

**Research Ethics**

All procedures in this study were reviewed and approved by the Research Ethics Committee of Poltekkes Kemenkes Riau, documented in approval letter No. LB.02.03/19/07/2024 and were conducted in compliance with the OECD Guidelines for the Testing of Chemicals and ARRIVE Guidelines 2.0. The choice of MSG dosage (0,25, 1, and 4 g/kg weight/day) was justified by its relevance to estimated human overconsumption levels, while the total sample size ($n = 24$) was considered sufficient to detect intergroup differences with acceptable statistical power while still adhering to the 3Rs principle (Replacement, Reduction, Refinement). To uphold ethical rigor, several measures were taken to minimize animal suffering, including acclimatization before treatment, provision of environmental enrichment, daily welfare monitoring, and the use of terminal anesthesia to prevent pain during sacrifice. Veterinary oversight was maintained throughout the study to ensure that animal health and welfare were prioritized.

## Result and Discussion

**Changes in ovarian morphology due to MSG exposure**

Microscopic evaluation of ovarian preparations revealed clear dose-dependent morphological changes between the control (K0) and treatment groups (K1–K3). In K0, ovarian structures displayed normal physiology, with a compact cortex, well-organized medulla, and evenly distributed follicles at every developmental stage, from primordial to Graafian follicles. No histopathological abnormalities, including edema, necrosis, or interstitial hemorrhage, were observed, indicating that the control animals maintained a healthy reproductive state throughout the study.

In contrast, chronic MSG exposure in K2 (moderate dose) and K3 (high-dose) groups led to progressive morphological degeneration. Follicular atrophy was evident, characterized by reduced follicle size, granulosa cell layer disorganization, and cytoplasmic vacuolization. Pyknotic nuclei and increased leukocyte infiltration were also observed, suggesting an inflammatory process. Furthermore, interstitial hemorrhage in these groups indicated microvascular fragility, likely induced by oxidative stress and subsequent capillary membrane damage. These findings collectively indicate that MSG triggers a chronic inflammatory response that may compromise oogenesis.

Quantitative analysis further supported this observation. As shown in Table 1, significant reductions were observed in the primary, secondary, and de Graaf follicle counts, with the most severe decline observed in the K3 group (secondary follicles: 3,1 ± 1,2; de Graaf follicles:

2,5 ± 0,9). These values differed significantly from those of K0 ($p < 0,01$ and $p < 0,05$, respectively). Interestingly, although the number of primordial follicles showed a decreasing trend, the difference was not statistically significant ($p > 0,05$). This suggests that early folliculogenesis remained relatively preserved within the 8-week exposure period, but later stages of follicle maturation were markedly vulnerable to chronic MSG intake.

The dose-dependent nature of these findings is crucial; low-dose exposure (K1) caused only mild, non-significant reductions, whereas moderate (K2) and high (K3) exposures produced pronounced, statistically significant effects. This pattern indicates a threshold phenomenon in which ovarian dysfunction becomes evident only at higher cumulative doses. Mechanistically, these changes may be linked to MSG-induced oxidative stress and subsequent apoptosis of granulosa cells, both of which are critical regulators of follicular development and survival. The strong correlation between MSG dose and follicle depletion reinforces this interpretation, highlighting MSG's potential role of MSG in accelerating ovarian aging and impairing reproductive capacity.

**Table 1.** Mean ± SD of ovarian follicle count by developmental stage across treatment groups

| Group | Follicle Primordial | Follicle Primer | Follicle Seconds | Follicle de Graaf |
|---|---|---|---|---|
| K0 | 12,5 ± 1,2 | 9,3 ± 1,5 | 7,8 ± 1,4 | 5,4 ± 1,1 |
| K1 | 11,9 ± 1,4 | 8,1 ± 1,7 | 6,2 ± 1,6 | 4,1 ± 1,3 |
| | p = 0,32 | p = 0,11 | p = 0,09 | p = 0,08 |
| K2 | 11,3 ± 1,1 | 6,7 ± 1,3 | 4,3 ± 1,5 | 3,2 ± 1,0 |
| | p = 0,07 | p = 0,04 | p = 0,008 | p = 0,03 |
| K3 | 10,8 ± 1,3 | 5,1 ± 1,6 | 3,1 ± 1,2 | 2,5 ± 0,9 |
| | p = 0,03 | p = 0,001 | p = 0,001 | p = 0,02 |

Note: Values were obtained from one-way ANOVA (for normally distributed data) or Mann–Whitney test (for non-normally distributed data) compared with the control group (K0). Normality was assessed using the Shapiro–Wilk test prior to ANOVA. $p < 0,05$ was considered statistically significant; n = 6 per group, female Wistar rats, 8 weeks old.

The gradual decrease in the number of functional follicles observed in this study underscores a clear dose-dependent relationship, in which higher MSG exposure corresponds to greater ovarian damage (Yang et al., 2023). This supports the hypothesis that MSG not only exerts systemic metabolic effects but also directly interferes with follicle maturation and oogenesis. Potential mechanisms include oxidative stress, tissue inflammation, and local hormonal imbalance in the ovaries (Taha et al., 2025).

Morphological degeneration identified in the ovaries of mice receiving moderate and high MSG doses, characterized by granulosa cell vacuolization, nuclear pyknosis, and interstitial hemorrhage, suggests a disruption of ovarian homeostasis and impaired follicular integrity. These changes are consistent with oxidative stress–mediated damage, which compromises cellular integrity and folliculogenesis. Similar patterns were reported by Othman and Suliman, who demonstrated that chronic MSG exposure elevated reactive oxygen species (ROS) levels in ovarian tissues, inducing lipid peroxidation and granulosa cell apoptosis (Othman & Suliman, 2020). Beyond oxidative stress, recent studies have highlighted that MSG can upregulate pro-inflammatory cytokines, such as TNF-α and IL-6, which further contribute to granulosa cell dysfunction and follicular atresia.

The marked decline in secondary and Graafian follicles observed in this study reflects an impaired ovarian reserve and ovulatory capacity. As these follicles represent the final pool available for ovulation, their depletion is directly linked to reduced reproductive potential. This finding aligns with that of Kayode et al., who showed that MSG interferes with follicle-stimulating hormone (FSH) signaling, thereby impairing follicle maturation. Furthermore, other animal studies have reported disrupted estrous cycles and reduced fertility rates following prolonged MSG exposure (Kayode et al., 2020). In humans, epidemiological data remain limited, but excessive dietary MSG intake has been associated with altered reproductive hormone levels and an increased risk of menstrual irregularities (Shi et al., 2019), suggesting the translational relevance of the present findings.

Interestingly, primordial follicles were relatively preserved during the eight-week treatment, indicating that the earliest stages of

folliculogenesis may be more resilient to MSG-induced toxicity in the short term than the later stages. However, chronic impairment in the later stages could eventually alter primordial follicle recruitment, leading to accelerated follicle depletion and premature ovarian insufficiency over longer exposures (Ducreux et al., 2023). This mechanism may mirror early ovarian aging, a condition increasingly recognized in women with high exposure to dietary additives and metabolic stress.

From a mechanistic perspective, MSG-induced ovarian toxicity likely involves multifactorial processes. First, excessive glutamate may disturb redox balance, enhancing mitochondrial ROS generation and activating apoptotic pathways through Bax/Bcl-2 signaling cascades. Second, ROS-driven lipid peroxidation may damage oocyte membranes, and oxidative DNA damage can compromise granulosa cell proliferation. Third, MSG disrupts hypothalamic-pituitary-ovarian (HPO) axis signaling, altering gonadotropin release and estrogen-progesterone balance (Hamdalla et al., 2023). These combined biochemical disturbances provide a plausible explanation for the structural and functional ovarian impairment observed.

These findings raise significant concerns regarding the reproductive health of women. Chronic consumption of MSG, particularly in populations with a high intake of processed foods, may contribute to subfertility, menstrual dysfunction, or even premature ovarian aging. Although the present study was conducted in mice, the parallels with human reproductive physiology suggest that further epidemiological and clinical research is urgently needed to evaluate the risks of long-term MSG exposure in women of reproductive ages.

**Cytometric Analysis and Oogenesis Indicators**

Cytometric analysis showed that exposure to MSG caused disturbance in oogenesis dynamics, characterized by a reduced proportion of oocyte cells in the late maturation phase (de Graaf follicle) and an increase in the number of degenerative germ cells. The K3 group showed karyolitic nucleus accumulation and cytoplasmic fragmentation, indicating an early apoptotic process. This difference was statistically significant ($p < 0,01$, based on the Kruskal–Wallis test).

Microscopic visualization supports these findings, with a description of the structure of the ovaries undergoing architectural disorganization, particularly in the granulosa and internal teka zones (Kadir et al., 2024). Figure 1 shows a representative comparison of ovarian morphology between the groups.

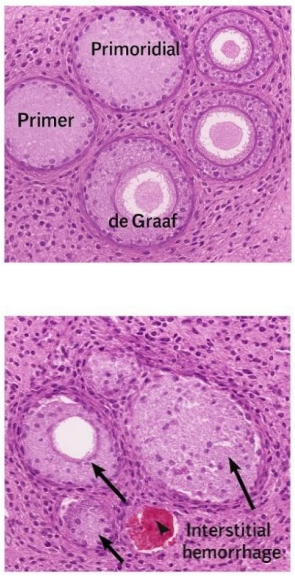



**Figure 1.** Histopathological examination of female rat ovaries.

(a) The control group showed normal follicular structures (primordial, primary, secondary, and de Graaf follicles), with no tissue abnormalities. (b) The high-dose MSG treatment group showed follicular degeneration, disorganization of the granulosa zone, and interstitial hemorrhage (indicated by arrows). Hematoxylin and eosin staining was performed at 400.

Cytometric findings showing a reduced proportion of oocytes in the late maturation phase (de Graaf follicles) and an increase in degenerative germ cells suggest that MSG disrupts the normal progression of oogenesis. The presence of karyolitic nuclei and cytoplasmic fragmentation in the K3 group is consistent with the initiation of apoptosis, indicating that MSG accelerates programmed cell death in ovarian tissue. These alterations are not only structural markers of damage but also functional indicators of impaired reproductive potential.

The significant differences identified using the Kruskal–Wallis test ($p < 0,01$) confirmed that these cytological changes were dose-dependent and not random. Similar observations were reported by Jozkowiak et al., who demonstrated that chronic exposure to dietary additives could induce architectural disorganization in granulosa and theca cells through oxidative stress and mitochondrial dysfunction. Granulosa

cell integrity is critical for follicle nourishment and estrogen production; therefore, their degeneration directly hampers follicular survival and maturation (Jozkowiak et al., 2022).

Microscopic evidence of interstitial hemorrhage and granulosa disorganization further supports the hypothesis that MSG exposure triggers inflammatory and vascular responses in the ovarian tissue. This combination of apoptotic signals, stromal inflammation, and disrupted follicular architecture provides a plausible explanation for the decreased oocyte maturation capacity observed in cytometric analyses (Watkins & Young, 2024; de Vasconcelos et al., 2020).

Overall, these results highlight that cytometric alterations can serve as sensitive biomarkers of ovarian toxicity. They underscored the broader implication that excessive MSG intake could impair reproductive fitness by reducing the pool of mature oocytes, thereby lowering the chances of successful ovulation and reducing fertility.

**Statistical Analysis of MSG Exposure on Ovarian Follicle Development**

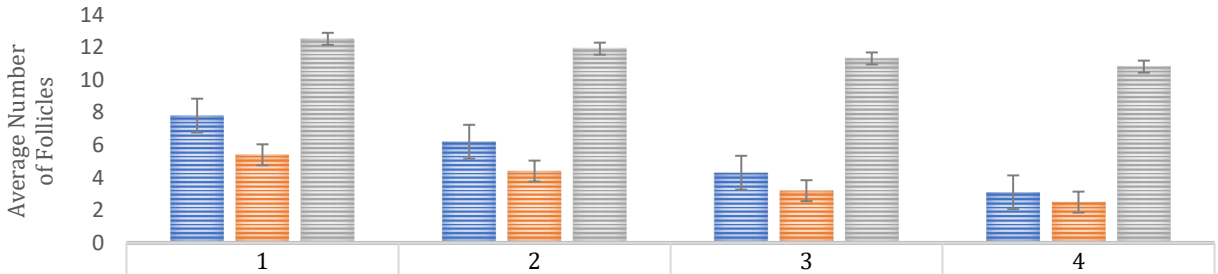
To evaluate the effect of Monosodium Glutamate (MSG) exposure on the number of ovarian follicles at each stage of development, inferential statistical analysis was performed using a One-Way Analysis of Variance test (Ogunmokunwa & Ibitoye, 2025). This test was used to determine whether there was a difference in the average number of follicles between the treatment groups (K0, K1, K2, and K3) administered different doses of MSG over an eight-week exposure period.

The results of the analysis showed a very statistically significant difference in the parameters of the number of secondary follicles and de Graaf follicles between the treatment groups, with a significance value of $p < 0,001$. These findings indicate that at least one group experienced morphological changes in the ovaries that were statistically different from those of the other groups due to MSG treatment.

To identify which groups showed significant differences, the Tukey Honestly Significant Difference (HSD) follow-up test was performed as a post hoc procedure. The Tukey test results revealed that the K2 (medium dose) and K3 (high dose) groups experienced a significant decrease in the number of follicles compared to the control (K0) and K1 (low dose) groups. This difference was most pronounced in the number of secondary follicles and Graafian follicles, which are important indicators of oocyte maturity and ovulation potential.

In contrast, no statistically significant differences were found between the K0 and K1 groups for most parameters measured. This showed that administration of MSG in low doses (0,25 g/kg body weight/day) for eight weeks did not cause significant histological effects on ovarian structure or follicular dynamics; therefore, it can be assumed that it was still within the biological tolerance limits of female mice for exposure to these food additives.

The results of these statistical tests support the dose-dependent effect pattern, in which an increase in MSG dose correlates with an increase in the degree of structural damage and a decrease in the number of functional follicles. A strong negative correlation was also found between MSG dose and de Graaf follicle count ($r = -0,72$; $p < 0,01$), which further reinforces that high levels of MSG may inhibit the late maturation phase of oogenesis.




| | 1 | 2 | 3 | 4 |
|---|---|---|---|---|
| Series1 | 7.8 | 6.2 | 4.3 | 3.1 |
| Series2 | 5.4 | 4.4 | 3.2 | 2.5 |
| Series3 | 12.5 | 11.9 | 11.3 | 10.8 |

**Figure 2.** Mean number of ovarian follicles by follicle type across MSG treatment groups (Mean ± SD)

These findings have important implications for the study of nutritional toxicology, given that secondary follicles and Graafian follicles are the main determinants of ovarian reproductive capacity (Caesar et al., 2024). Significant declines in both follicle types indicate the potential for ovulation inhibition and decreased fertility, even before systemic hormonal abnormalities occur. Therefore, these results reinforce the urgency of revisiting the safe consumption limits of MSG in long-term dietary patterns, particularly in the female reproductive-age population.

Statistical analysis clearly demonstrated that chronic MSG exposure exerts a dose-dependent negative effect on ovarian follicle development, particularly in the secondary and de Graaf stages. These two follicular stages are critical determinants of oocyte maturation and ovulation readiness; therefore, their significant reduction suggests an impaired reproductive capacity (Moghadam et al., 2022). The strong negative correlation between MSG dose and de Graaf follicle count further highlights that a higher MSG intake directly inhibits the final stages of oogenesis (Mondal et al., 2018).

The absence of significant differences between the control (K0) and low-dose (K1) groups implies that the ovaries can tolerate limited MSG exposure without major histological or functional alterations. However, the marked decline observed in the medium- and high-dose groups (K2 and K3) supports the notion that once MSG intake surpasses the biological tolerance threshold, it causes structural and functional ovarian damage in rats. These findings align with those of previous studies linking MSG exposure to oxidative stress, granulosa cell apoptosis, and disruption of local endocrine signaling in the ovaries (Askar et al., 2025; Liu et al., 2023).

From a reproductive toxicology perspective, the decrease in secondary and de Graaf follicles is particularly alarming, as it indicates that MSG may compromise the ovarian reserve and hinder the ovulatory process even prior to overt hormonal dysregulation. This finding reinforces the hypothesis that MSG toxicity is not only systemic but also directly localized in the ovarian tissue, possibly mediated by free radical accumulation, mitochondrial dysfunction, and inflammation of the follicular microenvironment (Chairunnisa, 2022).

The findings of this study underscore the dose-dependent reproductive toxicity of MSG and provide mechanistic insights into how dietary exposure can progressively impair ovarian folliculogenesis. This evidence highlights the need for further translational research to determine safe exposure thresholds in humans and to re-evaluate the current dietary guidelines regarding MSG consumption, particularly in women of reproductive age.

## Conclusion

This study demonstrated that chronic exposure to Monosodium Glutamate (MSG), particularly at moderate to high doses, significantly disrupted ovarian morphology and follicular dynamics in female rats, as evidenced by a reduction in functional follicles and signs of germ cell degeneration.

These findings indicate the potential reproductive toxicity of MSG and underscore the need for careful evaluation of its long-term dietary intake, especially among women of reproductive age, who are more susceptible to hormonal and ovarian dysfunction. From a public health perspective, the results highlight the importance of establishing clearer intake guidelines and regulatory reviews of MSG consumption limits, supported by awareness campaigns to educate the community about the risks of excessive use.

Future research should validate these effects in human populations, explore the molecular mechanisms underlying MSG-induced ovarian toxicity, and develop nutrition-based preventive strategies. By integrating laboratory evidence with preventive policies and public education, this study provides a scientific foundation for safeguarding reproductive health and promoting healthier dietary practices at both the individual and community levels.

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## References

Al-Otaibi, A. M., Emam, N. M., Elabd, H. K., & Esmail, N. I. (2022). Toxicity of monosodium glutamate intake on different tissues induced oxidative stress: A review. *Journal of Medical and Life Science, 4*(4), 68–81. https://doi.org/10.21608/jmals.2022.264345

Al-Suhaimi, E. A., Khan, F. A., & Homeida, A. M. (2022). Regulation of male and female reproductive function. In *Emerging concepts in endocrine structure and functions* (pp. 287–347). Springer. https://doi.org/10.1007/978-3-030-93439-7_13

Askar, M. E., Abdel-Maksoud, Y. T., Shaheen, M. A., & Eissa, R. G. (2025). Ameliorating monosodium glutamate-induced testicular dysfunction by modulating steroidogenesis, oxidative stress, inflammation, and apoptosis: Therapeutic role of hesperidin. *Biochemical and Biophysical Research Communications, 771,* 152032. https://doi.org/10.1016/j.bbrc.2024.152032

Banerjee, A., Mukherjee, S., & Maji, B. K. (2021). Worldwide flavor enhancer monosodium glutamate combined with high lipid diet provokes metabolic alterations and systemic anomalies: An overview. *Toxicology Reports, 8,* 938–961. https://doi.org/10.1016/j.toxrep.2021.04.020

Caesar, J., Widjiati, W., Herupradoto, E. B. A., Sukmanadi, M., Madyawati, S. P., Plumeriastuti, H., & Luqman, E. M. (2024). Effect of curcumin nanoparticles on the number of preantral and antral follicles of white rats (*Rattus norvegicus*) exposed to carbon black. *Open Veterinary Journal, 14*(12), 3309–3316. https://doi.org/10.5455/OVJ.2024.v14.i12.4

Chairunnisa, N. I. (2022). The effect of green tea extract (*Camellia sinensis*) on the number of ovarian follicles of female white rat (*Rattus norvegicus*) exposed to MSG (monosodium glutamate): A literature review. *Manganite: Journal of Chemistry and Education, 1*(1), 8–14. https://doi.org/10.56709/manganite.v1i1.4

Das, P. K., Mukherjee, J., & Banerjee, D. (2023). Female reproductive physiology. In *Textbook of veterinary physiology* (pp. 513–568). Springer. https://doi.org/10.1007/978-981-99-7357-6_24

de Vasconcelos, G. L., Maculan, R., da Cunha, E. V., Silva, A. W. B., Batista, A. L. S., Donato, M. A. M., Peixoto, C. A., Silva, J. R. V., & de Souza, J. C. (2020). Antral follicular count and its relationship with ovarian volume, preantral follicle population and survival, oocyte meiotic progression and ultrastructure of in vitro matured bovine cumulus–oocyte complexes. *Zygote, 28*(6), 495–503. https://doi.org/10.1017/S0967199420000125

Ducreux, B., Ferreux, L., Patrat, C., & Fauque, P. (2023). Overview of gene expression dynamics during human oogenesis/folliculogenesis. *International Journal of Molecular Sciences, 25*(1), 33. https://doi.org/10.3390/ijms25010033

Fineschi, B. (2022). Selection of competent oocytes by morphological features: Can an artificial intelligence-based model predict oocyte quality? *Journal of Assisted Reproduction and Genetics, 39,* 1403–1414. https://doi.org/10.1007/s10815-022-02524-9

Hamdalla, H. M., Ahmed, R. R., Galaly, S. R., & Abdul-Hamid, M. (2023). Effects of quercetin on ovarian toxicity induced by dietary monosodium glutamate. *Cell and Tissue Biology, 17*(5), 543–556. https://doi.org/10.1134/S1990519X23050112

Jozkowiak, M., Piotrowska-Kempisty, H., Kobylarek, D., Gorska, N., Mozdziak, P., Kempisty, B., Rachon, D., & Spaczynski, R. Z. (2022). Endocrine disrupting chemicals in polycystic ovary syndrome: The relevant role of the theca and granulosa cells in the pathogenesis of the ovarian dysfunction. *Cells, 12*(1), 174. https://doi.org/10.3390/cells12010174

Kadir, E. R., Yakub, A. D., Ojulari, L. S., Hussein, A. O., Lawal, I. A., Jaji-Sulaimon, R., & Ajao, M. S. (2024). Cytoarchitectural differences in reproductive organs of some polycystic ovary-like induced animal models. *Tissue and Cell, 89,* 102456. https://doi.org/10.1016/j.tice.2024.102456

Kayode, O. T., Bello, J. A., Oguntola, J. A., Kayode, A. A. A., & Olukoya, D. K. (2023). The interplay between monosodium glutamate (MSG) consumption and metabolic disorders. *Heliyon, 9*(9), e21055. https://doi.org/10.1016/j.heliyon.2023.e21055

Kayode, O. T., Rotimi, D. E., Kayode, A. A. A., Olaolu, T. D., & Adeyemi, O. S. (2020). Monosodium glutamate (MSG)-induced male reproductive dysfunction: A mini review. *Toxics, 8*(1), 7. https://doi.org/10.3390/toxics8010007

Kesherwani, R., Bhoumik, S., Kumar, R., & Rizvi, S. I. (2024). Monosodium glutamate even at low dose may affect oxidative stress, inflammation and neurodegeneration in rats. *Indian Journal of Clinical Biochemistry, 39*(1), 101–109. https://doi.org/10.1007/s12291-023-01127-1

Liu, S., Jia, Y., Meng, S., Luo, Y., Yang, Q., & Pan, Z. (2023). Mechanisms of and potential medications for oxidative stress in ovarian granulosa cells: A review. *International Journal of Molecular Sciences, 24*(11), 9205. https://doi.org/10.3390/ijms24119205

Moghadam, A. R. E., Moghadam, M. T., Hemadi, M., & Saki, G. (2022). Oocyte quality and aging. *JBRA Assisted Reproduction, 26*(1), 105–115. https://doi.org/10.5935/1518-0557.20220007

Mondal, M., Sarkar, K., Nath, P. P., & Paul, G. (2018). Monosodium glutamate suppresses the female reproductive function by impairing the functions of ovary and uterus in rat. *Environmental Toxicology, 33*(2), 198–208. https://doi.org/10.1002/tox.22507

Nazam, N., Jabir, N. R., Ahmad, I., Alharthy, S. A., Khan, M. S., Ayub, R., & Tabrez, S. (2023). Phenolic acids-mediated regulation of molecular targets in ovarian cancer: Current understanding and future perspectives. *Pharmaceuticals, 16*(2), 274. https://doi.org/10.3390/ph16020274

Ogunmokunwa, A. E., & Ibitoye, B. O. (2025). Monosodium glutamate (MSG) exposure induced oxidative stress and disrupted testicular hormonal regulation, exacerbating reproductive dysfunction in male Wistar rats. *Endocrine and Metabolic Science, 17,* 100226. https://doi.org/10.1016/j.endmts.2025.100226

Osawa, Y. (2022). The socio-cultural reception of MSG (monosodium glutamate) in Thailand. In *Making food in local and global contexts: Anthropological perspectives* (pp. 55–68). Springer. https://doi.org/10.1007/978-3-030-99691-3_4

Othman, S. Al, & Suliman, R. (2020). How pectin play a role in histological changes by monosodium glutamate (MSG) in the ovary of mice? *Annals of R.S.C.B., 24*(4), 9020–9030. http://annalsofrscb.ro

Reid, K., & Price, B. (2023). *Fat, stressed, and sick: MSG, processed food, and America's health crisis*. Rowman & Littlefield. https://rowman.com/ISBN/9781538169601

Rinninella, E., Cintoni, M., Raoul, P., Mora, V., Gasbarrini, A., & Mele, M. C. (2021). Impact of food additive titanium dioxide on gut microbiota composition, microbiota-associated functions, and gut barrier: A systematic review of in vivo animal studies. *International Journal of Environmental Research and Public Health, 18*(4), 2008. https://doi.org/10.3390/ijerph18042008

Sarmento, E. B., Sassone, L. M., Pinto, K. P., Ferreira, C. M. A., da Fidalgo, T. K. S., Lopes, R. T., Alves, A. T. N. N., Freitas-Fernandes, L. B., Valente, A. P., & Neves, R. H. (2025). Evaluation of a potential bidirectional influence of metabolic syndrome and apical periodontitis: An animal-based study. *International Endodontic Journal, 58*(5), 517–530. https://doi.org/10.1111/iej.14286

Taha, M., Ali, L. S., El-Nablaway, M., Ibrahim, M. M., Badawy, A. M., Farage, A. E., Ibrahim, H. S. A., Zaghloul, R. A., & Hussin, E. (2025). Multifaceted impacts of monosodium glutamate on testicular morphology: Insights into pyroptosis and therapeutic potential of resveratrol. *Folia*

*Morphologica, 84*(1), 151–166. https://doi.org/10.5603/FM.a2024.0121

Watkins, J. C., & Young, R. H. (2024). Nonneoplastic disorders of the ovary. In *Pathology of the ovary, fallopian tube and peritoneum* (pp. 35–58). Springer. https://doi.org/10.1007/978-3-031-42642-6_3

Wijayasekara, K. N., & Wansapala, J. (2021). Comparison of a flavor enhancer made with locally available ingredients against commercially available monosodium glutamate. *International Journal of Gastronomy and Food Science, 23,* 100286. https://doi.org/10.1016/j.ijgfs.2020.100286

Yang, L., Gao, Y., Gong, J., Peng, L., El-Seedi, H. R., Farag, M. A., Zhao, Y., & Xiao, J. (2023). A multifaceted review of monosodium glutamate effects on human health and its natural remedies. *Food Materials Research, 3*(1). https://doi.org/10.48129/kjs.v3i1.231

Zhuang, H., Liu, X., Wang, H., Qin, C., Li, Y., Li, W., & Shi, Y. (2021). Diagnosis of early stage Parkinson's disease on quantitative susceptibility mapping using complex network with one-way ANOVA F-test feature selection. *Journal of Mechanics in Medicine and Biology, 21*(5), 2140026. https://doi.org/10.1142/S0219519421400264