

Research Paper

Dietary Effects of Acetaminophen Tenderized Meat on Reproductive Hormones, Gonadal Weight and Histology in Female Albino Mice (*Mus musculus*)

Germaine Akinola Ogunwole

Department of Animal and Environmental Biology, School of Life Science, Federal University of Technology, Akure. P.M.B 704, Ondo, Nigeria

Corresponding: gaogunwole@futa.edu.ng

ARTICLE INFO

Article history:

Received: 29-08-2026

Revised: 05-10-2026

Accepted: 05-10-2026

Available online: 17-10-2026

Keywords:

Acetaminophen, meat tenderization, reproductive toxicity, ovarian damage, Swiss mice, hormonal imbalance

Abstract

The misuse of Acetaminophen (ACT) by homes and food sellers as a cheap and fast meat tenderizer is becoming a huge concern despite its potential health risks. This study focused on examining the reproductive toxicity of meat tenderized with ACT using female Swiss mice (*Mus musculus*). For 35 days, 30 sexually mature mice were divided into five groups; the negative control group received standard rat feed only, another control group received standard feed (40 g) + untreated meat (20 g), while the treated groups were fed standard feed (40 g) + ACT-tenderized meat (20 g), and then sacrificed at days 15, 25, and 35 respectively. Follicle stimulating hormone (FSH), luteinizing hormone (LH), progesterone, and estradiol were measured in serum, and ovarian tissues were weighed and examined for histopathology alterations. Statistical evaluation was performed using one-way ANOVA, with Tukey's post hoc test applied for mean separation. The serum analysis showed a significant elevation in estradiol levels in mice exposed to ACT-tenderized meat for 25 days (8.49 mIU/ml) and 35 days (8.01 mIU/ml), respectively. Furthermore, A significant reduction in ovarian weight was observed in all treated groups throughout the study period. The ovarian weight decreased from 0.71– 0.74 mg in the control groups to approximately 0.10–0.11 mg in ACT-tenderized meat-exposed mice, with the least reduction recorded in the 15-day exposure group (0.103 mg). Histological examination of the treated groups showed disruption of normal architecture and degenerative alterations in ovarian follicles. These results imply that eating meat tenderized with acetaminophen may provide long term serious concerns to the reproductive health of women. Therefore, it is advised that public health awareness and regulatory enforcement be implemented to stop the abuse of drugs in food processing.

1. Introduction

Meat tenderness plays a crucial role in determining the sensory quality of meat, directly influencing consumer satisfaction, preferences, purchasing decisions, and the overall marketability of meat products (Madhusankha and Thilakarathna, 2020). It is also often regarded as the most critical sensory attribute, even outweighing factors such as appearance, flavor, and juiciness (Azmi et al., 2023). Over the years, various methods to enhance meat tenderness have been explored, often ranging from mechanical and chemical interventions to the application of exogenous proteases (Bekhit et al., 2014). However, the choice of method is frequently influenced by factors such as energy consumption, cost, and the availability of tenderizing agents. In developing economies, where economic constraints are significant, food vendors tend to favor approaches that minimize energy costs and rely on readily available, low-cost materials. One such commonly used method is the deployment of chemical agents, including acetaminophen (ACT), to achieve the desired tenderness while keeping expenses manageable.

Acetaminophen (ACT), chemically named N-acetyl-para-aminophenol (APAP) and commonly called paracetamol, has been widely used as a fever and pain reliever for both acute and chronic conditions (Hashem et al., 2023). ACT is sometimes misused by food vendors as a meat tenderizer due to its ability to accelerate the softening of tough meat, especially in resource limited settings. This tenderizing effect is thought to occur through the activation of biochemical pathways similar to natural postmortem processes. Specifically, when ACT is heated during cooking, it may trigger apoptotic signaling in muscle cells, leading to the activation of caspase enzymes like caspase-3 and caspase-9 (Shi et al, 2021). These enzymes play a role in breaking down muscle proteins by promoting the activity of calpains proteolytic enzymes that degrade structural proteins such as titin, nebulin, and troponin-T, which are responsible for meat toughness (Ezugwu et al., 2023). This process is enhanced by the inhibition of calpastatin, which is the natural inhibitor of calpains. Thus, further increasing proteolysis and resulting in softer, more palatable meat (Ezugwu et al., 2023). However, this chemical shortcut comes at a high risk because paracetamol breaks down under heat into toxic substances such as 4-aminophenol and N-acetyl-p-benzoquinone imine (NAPQI), which are known to cause liver and kidney damage (Moles et al., 2018). These toxic byproducts are especially dangerous when consumed repeatedly or in large quantities, making this method of meat tenderization a serious public health risk.

The reproductive system is highly sensitive to external environmental factors, and exposure to toxic substances, even at low levels, can have significant and lasting effects. Studies have shown that certain pharmaceutical agents, environmental pollutants, food additives inclusive can disrupt reproductive processes by interfering with hormone regulation, impairing gamete production, or causing developmental abnormalities (Kadhim, 2024; Okuthe et al., 2025). The female reproductive system, particularly the ovary, is regulated by a finely balanced network of hormones, primarily estrogen, progesterone, follicle-stimulating hormone (FSH), and luteinizing hormone (LH). These hormones coordinate follicular development, ovulation, and corpus luteum formation, ensuring normal reproductive function (Rosner et al., 2024).

To evaluate how such exposures influence fertility, pregnancy outcomes, and offspring development, Swiss mice are widely used because they share many fundamental anatomical and hormonal features with humans, making them useful models for studying reproductive biology (Perlman, 2016). These similarities make them a reliable model for investigating chemical effects on reproductive performance. Commonly assessed endpoints include fertility indices, circulating hormone levels, and morphological or functional abnormalities in their gonads or progeny (ECETOC, 2022). Furthermore, any disruption to this endocrine equilibrium whether through exogenous chemicals or internal physiological imbalances can alter ovarian morphology and function. Histologically, the ovary exhibits a cyclic pattern of structural changes that mirror hormonal fluctuations. For instance, cortex contains follicles at various stages of maturation, from primordial to Graafian, while the medulla houses connective tissue, blood vessels, and nerves (Williams et al., 2012). Exposure to endocrine-disrupting compounds (EDCs) has been associated with altered follicular dynamics, including increased follicular atresia (Tricotteaux-Zarqaoui et al., 2024), degeneration of granulosa cells, and thickening of the tunica albuginea (Wu et al., 2023). Such alterations often correspond to reduced steroidogenic activity, impaired ovulation, and subfertility (Tricotteaux-Zarqaoui et al., 2024).

Across multiple species, direct exposure to ACT have been reported to give rise to reproductive and developmental disruptions. In Swiss CD-1 mice, high-dose dietary exposure reduced litter size and number, and while birth weight was unaffected, it also impaired postnatal growth (Reel et al., 1992). ACT has also been implicated in the disruption of pre-implantation embryonic development by inhibiting ribonucleotide reductase, arresting the cell cycle, and causing early embryonic loss in both mouse and human embryos (Nielsen et al., 2025). Moderate to high doses of ACT have also been reported to increase adverse pregnancy outcomes and altered blood phenotypes in dams and fetuses, inhibiting fetal hippocampal, bone, and organ development. This is in addition to promoting hepatic lipid synthesis, renal inflammation, and adrenal hormone production (Chen et al., 2024). In male reproductive systems, high dose or long term exposure to ACT caused reversible testicular damage and impaired semen quality by suppressing testosterone synthesis, inducing oxidative stress, and provoking spermatocyte apoptosis (Banihani, 2017; Lee et al., 2023). Prenatal exposure can also disrupt gonadal function in offspring, reducing ovarian weight and cyclicity and altering ovarian immune cells, while in males, it impairs testis size and spermatogenesis (Bazzano et al., 2023).

Despite the well documented concern over the practice of using ACT as a meat tenderizing agent, there is little to no existing literature specifically addressing the reproductive toxicity of consuming such meat. Most available studies have focused on its hepatotoxic and nephrotoxic effects, especially at acute and sub-acute concentrations. Thus, leaving critical gaps in knowledge concerning its impact on reproductive hormones and organs. This lack of scientific data makes it difficult to assess the full risk of this food safety issue. This study is therefore essential in filling that knowledge gap by examine whether dietary exposure to ACT-tenderized meat can lead to hormonal imbalance and structural damage to the ovaries. Given the importance of estrogen and progesterone in female reproductive health, any disruption in their levels could have significant implications on fertility.

2. Materials and Methods

2.1 Housing Conditions

The housing was prepared in a dedicated animal house domiciled in the Department of Biology, School of Life Sciences, Federal University of Technology. A total of five cages (45 X 25 X 25 cm) were prepared for the animals; it was cleaned, lined with wood shavings and a constant supply of water was made available.

2.2 Animal Collection, Grouping and Housing

A total of 30 mature female Swiss mice (*Mus musculus*), weighing between 20 - 28 g were obtained from University Teaching Hospital, Ibadan. The animals were subjected to 2 weeks acclimatization period. They were then weighed and grouped according to weight into four groups (n=5 per group). The grouping include:

Group I (Control): Fed normal rat feed only.

Group II (Meat Control): Fed standard feed + tenderized meat only without acetaminophen.

Group III: Treated with acetaminophen meat mixture, sacrificed on day 15.

Group IV: Treated with acetaminophen meat mixture, sacrificed on day 25.

Group V: Treated with acetaminophen meat mixture, sacrificed on day 35.

2.3 Meat Preparation with Acetaminophen and Feeding Regime

Acetaminophen tablets (500 mg) were obtained from a registered pharmacy store, and the red meat was obtained from Adebowale market, in Akure South Local Government Area. Ondo State. Nigeria. Before commencement of the experiment, roadside food vendors and individuals were quizzed on the average quantity of ACT used in their meat tenderization processes, and the average quantity of ACT deployed per kg of meat amounted to 300 mg. Subsequently, the tenderization of the meat with ACT was prepared by placing 500 mg the tablets in 2.5 L of distilled water with 2 kg of red meat, and boiled for 15 minutes. The tenderized meat was allowed to cool to room temperature, then manually pounded into smaller pieces, before being stored in a refrigerator maintained under constant electricity supply to ensure preservation.

2.3.1 Feeding Regimen

In a free feeding system, mice in Groups III–V were fed with 60 g of a feed mixture containing 20 g of ACT-treated meat and 40 g of normal feed. While Group I (Negative control) received 60 g of normal feed, and the Group II (Non-ACT tenderized meat control) received with 60 g of a feed mixture containing 20 g of non-ACT-treated meat and 40 g of normal feed. Furthermore, mice were fed daily and the feeding continued daily for 35 days.

2.4 Sample Collection

Prior to sample collection at the end of their respective treatment groups (days 15, 25, and 35), the laboratory bench and other materials (slaughter slab, scissors, and forceps) was wiped clean with 70% ethanol to ensure aseptic conditions, prevent cross-contamination of samples, and

maintain the integrity and reliability of experimental results. Following ethical approval and standard animal handling guidelines, the mice were humanely euthanized by cervical dislocation, performed by trained personnel to ensure rapid loss of consciousness and minimize distress. The animals were then positioned on a dissection platform, and the abdominal cavity was carefully opened using sterile scissors and forceps to expose the internal organs. Blood samples were collected through cardiac puncture using a sterile 2 ml syringe and transferred into sample tubes for subsequent hormonal assays. Following blood collection, the ovaries were carefully excised, rinsed, weighed and immediately fixed in 10% buffered formalin for histopathological examination.

2.5 Hormone Assay

2.5.1 Estradiol

Serum estradiol concentrations were determined using a competitive enzyme-linked immunosorbent assay (ELISA) kit. Briefly, 25 μL of calibrator, control, or mouse serum sample was added to the designated wells, followed by the addition of 50 μL of estradiol biotin reagent. The plate was gently mixed for 20–30 seconds, covered, and incubated at room temperature for 30 minutes. Subsequently, 50 μL of estradiol enzyme reagent (horseradish peroxidase conjugate) was added to each well, mixed gently, and incubated for an additional 90 minutes at room temperature.

Following incubation, the wells were aspirated and washed three times with 350 μL of diluted wash buffer to remove unbound reagents. After washing, 100 μL of TMB substrate was added to each well, and the plate was incubated in the dark at room temperature for 20 minutes. The enzymatic reaction was terminated by the addition of 50 μL of stop solution (0.5 M H_2SO_4) to each well, with gentle mixing for 15–20 seconds. Absorbance was measured within 15 minutes at 450 nm, with a reference wavelength of 620–630 nm, using a microplate reader.

2.5.2 Luteinizing Hormone (LH)

Serum LH concentrations were estimated using a chemiluminescent immune-enzymometric assay (CLIA). Briefly, 50 μL of calibrator, control, or Swiss mouse serum sample was added to each designated well, followed by 100 μL of LH tracer reagent containing enzyme-labeled and biotinylated antibodies. The plate was gently mixed for 20–30 seconds, covered, and incubated at room temperature for 45 minutes. After incubation, the wells were aspirated and washed five times with 350 μL of diluted wash buffer to remove unbound reagents. Subsequently, 100 μL of the freshly prepared signal reagent (equal parts Signal A and Signal B) was added to each well. The plate was incubated in the dark at room temperature for 5 minutes. The chemiluminescent reaction was then measured using a microplate luminometer, and the relative light units (RLUs) were recorded for each well within 30 minutes of signal addition.

2.5.3 Follicle Stimulating Hormone

Serum FSH concentrations were evaluated using a commercial ELISA kit based on the sandwich immunoassay principle (streptavidin-coated 96-well microplate, enzyme reagent, calibrators, wash buffer, TMB substrates A and B, and stop solution, 1 N HCl). All reagents were stored at 2 – 8 °C and protected from prolonged heat or light exposure, in

accordance with the manufacturer's instructions. Prior to assay, serum samples obtained from Swiss mice were thawed at room temperature and mixed gently. The procedure was carried out as follows: 50 μ L of calibrator, control, or test serum was pipetted into the designated wells, after which 100 μ L of the enzyme conjugate was added. The plate was gently agitated for 20–30 seconds, covered, and incubated at room temperature (20–27 °C) for 60 minutes. After incubation, the contents of the wells were aspirated, and the wells were washed three times with 350 μ L of diluted wash buffer, ensuring thorough removal of unbound materials. Subsequently, 100 μ L of the working TMB substrate was added to each well. The plate was incubated for 15 minutes at room temperature, without agitation, to allow for color development. The reaction was terminated by adding 50 μ L of stop solution (1 N HCl) to each well and gently mixing for approximately 15–20 seconds. Optical density (OD) was read within 30 minutes at a primary wavelength of 450 nm with a reference filter of 620–630 nm using a microplate reader.

2.5.4 Progesterone

Serum progesterone concentrations were measured using a competitive enzyme immunoassay principle. Briefly, 25 μ L of calibrator, control, or Swiss mouse serum sample was pipetted into designated wells, followed by the addition of 50 μ L of Progesterone enzyme reagent and 50 μ L of biotinylated anti-progesterone antibody. The plate was gently mixed for 10–20 seconds, covered, and incubated for 60 minutes at room temperature. Following incubation, the wells were aspirated, and washed three times with 350 μ L of diluted wash buffer to remove unbound reagents. Next, 100 μ L of TMB substrate solution was added to each well and incubated at room temperature for 20 minutes without agitation, allowing color development. The enzymatic reaction was stopped by adding 50 μ L of stop solution (1 N H₂SO₄) to each well and gently mixing for 15–20 seconds. Optical density (OD) was measured at a primary wavelength of 450 nm with a reference wavelength of 620–630 nm within 15 minutes of stopping the reaction.

2.6 Histopathology

The harvested ovaries of the mice were fixed in 10% formalin to stop them from decomposing. For one and a half hours, they were dehydrated in various alcohol percentages (50, 70%, 80%, 90%, and 100%). Following dehydration, they were cleared with 100% xylene and allowed to sit for one or two hours to eliminate any remaining alcohol. The cleared tissues were then infiltrated with paraffin wax in an oven maintained at 50–70°C. The wax was changed every hour for a total of two hours to ensure complete penetration of the tissue and removal of any residual xylene. Finally, they were placed in a cassette using a tissue embedding mold and allowed to cool with ice. A 7 micron microtome was used to segment the embedded organs, and haematoxylin-Eosin staining was applied. Tap water was used to remove the excess stain. After clearing in xylene, cover slips were applied, and Canada balsam or DPX was added. After being baked at 40°C, the preparations were checked by a professional and photographed using a microscope equipped with a digital camera that was linked to a computer system.

2.7 Data Analysis

The statistical analysis was done with the help of graphpad prism version 9, and One way ANOVA was used to analyze the data. Additionally, the Tukey's post-hoc test was used to separate the means at significant level $P < 0.05$.

3. Results

3.1 Hormonal Analysis

There was a significant increase in the level of estradiol in mice after days 25 (8.49 mlU/ml) and 35 (8.01 mlU/ml), when compared with both control groups (7.05 -7.54 mlU/ml) (Figure 1). The lowest estradiol level was observed in mice fed with ACT tenderized meat after 15 days (7.02 mlU/ml), while the highest was observed on day 25 (8.49 mlU/ml). The LH level in the treated mice was not significantly different from that observed in the various control groups. The LH in the controls ranged between 1.47 -1.53 mlU/ml, while that of the exposed groups were between 1.55 – 2.09 (mlU/ml), with the highest level observed after day 35. The result of the follicle stimulating hormone (mlU/ml) is presented in Figure 3. Just like what was observed with the LH, FSH level in the treated mice did not differ significantly from the control group. The FSH in the controls ranged between 2.03 - 2.05 mlU/ml, while that of the exposed groups were between 2.04 – 2.44 mlU/ml, with the highest level observed after day 35 as well. In like manner with the LH and FSH hormone, the progesterone hormone in the treated rats was not significantly different from the levels measured in the controls (Figure 4). The progesterone hormone in the control ranged between 4.65 -4.75 mlU/ml, while that in the treated group ranged between 4.64 – 4.92 mlU/ml.

3.2 Gonadal Weight

The ovarian weight of the treated group revealed significant reduction throughout the study period (Figure 5). The ovarian weight reduced from 0.71 – 0.74 mg in the control groups to approximately 0.10 - 0.11 g in all the treated groups. Furthermore, the least reduction was observed in the group treated for 15 days (0.103 mg).

3.3 Ovary Histology Analysis

Histological examination of ovarian tissues revealed distinct differences between the control groups and ACT-treated groups. The results are shown in plates 1a -1i. Ovarian histopathology assessment of the control groups displayed similar pattern, with the ovaries showing normal architecture with clearly visible primordial follicles (PRF) and antrum (ANT) (Plate 1a). Furthermore, the stroma appeared intact with no evidence of inflammation, fibrosis, or degenerative changes, indicating normal ovarian function (Plate 1a). Mice exposed for 15 days had their ovarian tissue displaying early pathological alterations. One of such alterations were the presence of haemorrhage (Plate 1c), along with degeneration of the zona granulosa (DG) (Plate 1d). With increasing exposure on day 25, observable incidences of haemorrhage (H) (Plate 1e), and inflammations (INF) around the oocyte were recorded (Plate 1f). More pronounced degenerative changes were recorded in group exposed for 35 days. Some of these notable ovarian alterations were atrophy (AT) in primary oocyte (Plate 1f), and fibrosis (F) in the ovary (Plate 1f). Furthermore, the most frequent damage that were recorded were haemorrhage across multiple sections of the vary (Plate 1h and 1i).

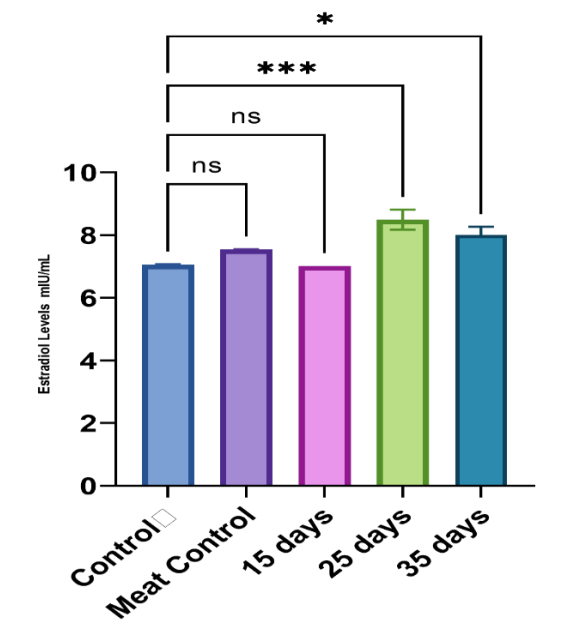


Figure 1. Estradiol levels in Female Mice fed with Acetaminophen Tenderized Meat.
Note: Stars (*) shows level of significance of the treated relative to the control. While NS, connotes no significant difference between the treated and the control

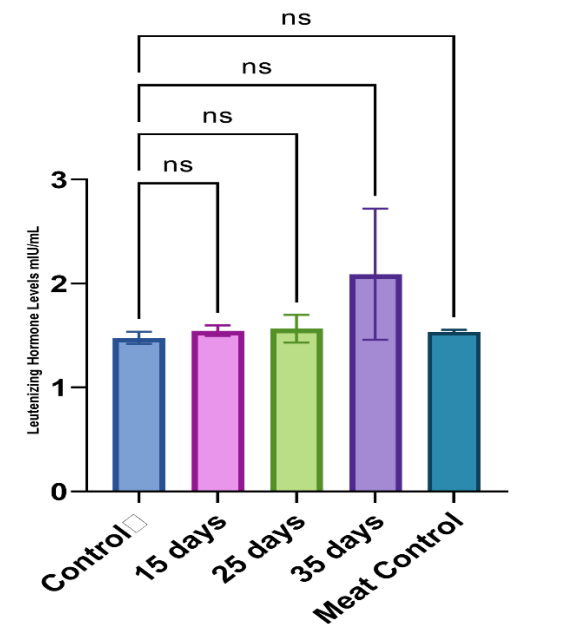


Figure 2. Luteinizing Hormone levels in Female Mice fed with Acetaminophen Tenderized Meat.
Note: Stars (*) shows level of significance of the treated relative to the control. While NS, connotes no significant difference between the treated and the control.

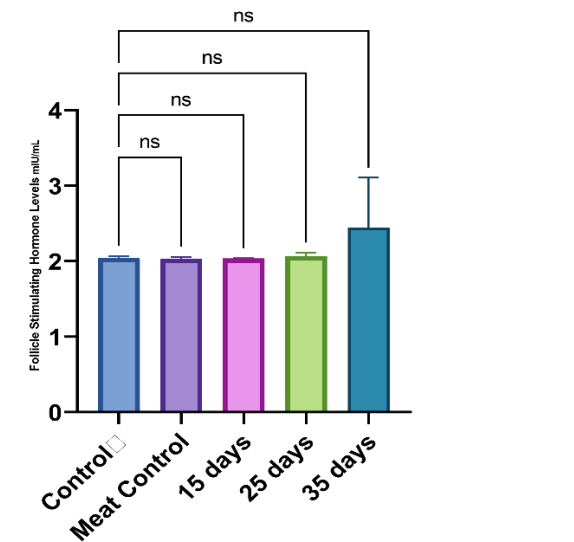


Figure 3. Follicle Stimulating Hormone levels in Female Mice fed with Acetaminophen Tenderized Meat.
Note: Stars (*) shows level of significance of the treated relative to the control. While NS, connotes no significant difference between the treated and the control.

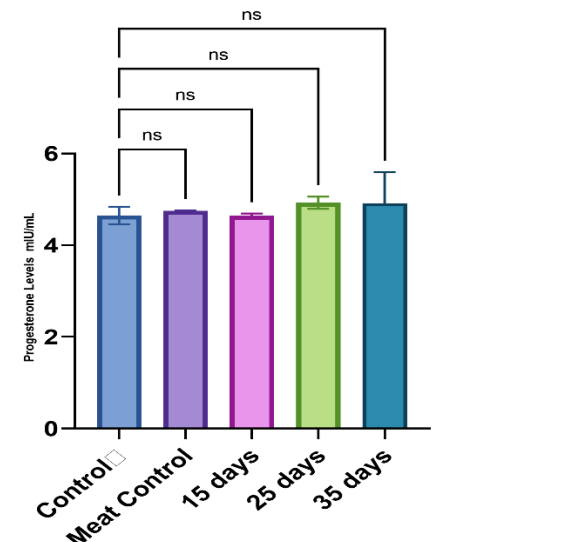


Figure 4. Progesterone levels in Female Mice fed with Acetaminophen Tenderized Meat.
Note: Stars (*) shows level of significance of the treated relative to the control. While NS, connotes no significant difference between the treated and the control.

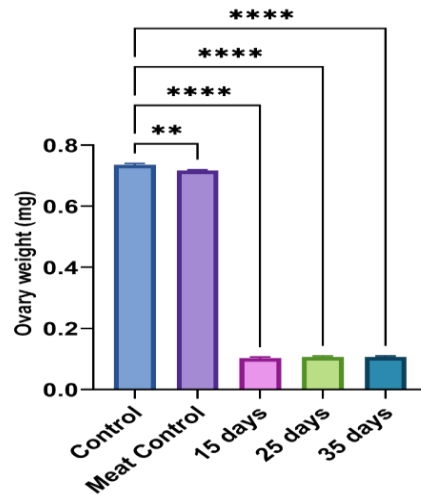


Figure 5. Ovary Weight in Female Mice fed with Acetaminophen Tenderized Meat.

Note: Stars (*) shows level of significance of the treated relative to the control. While NS, connotes no significant difference between the treated and the control.

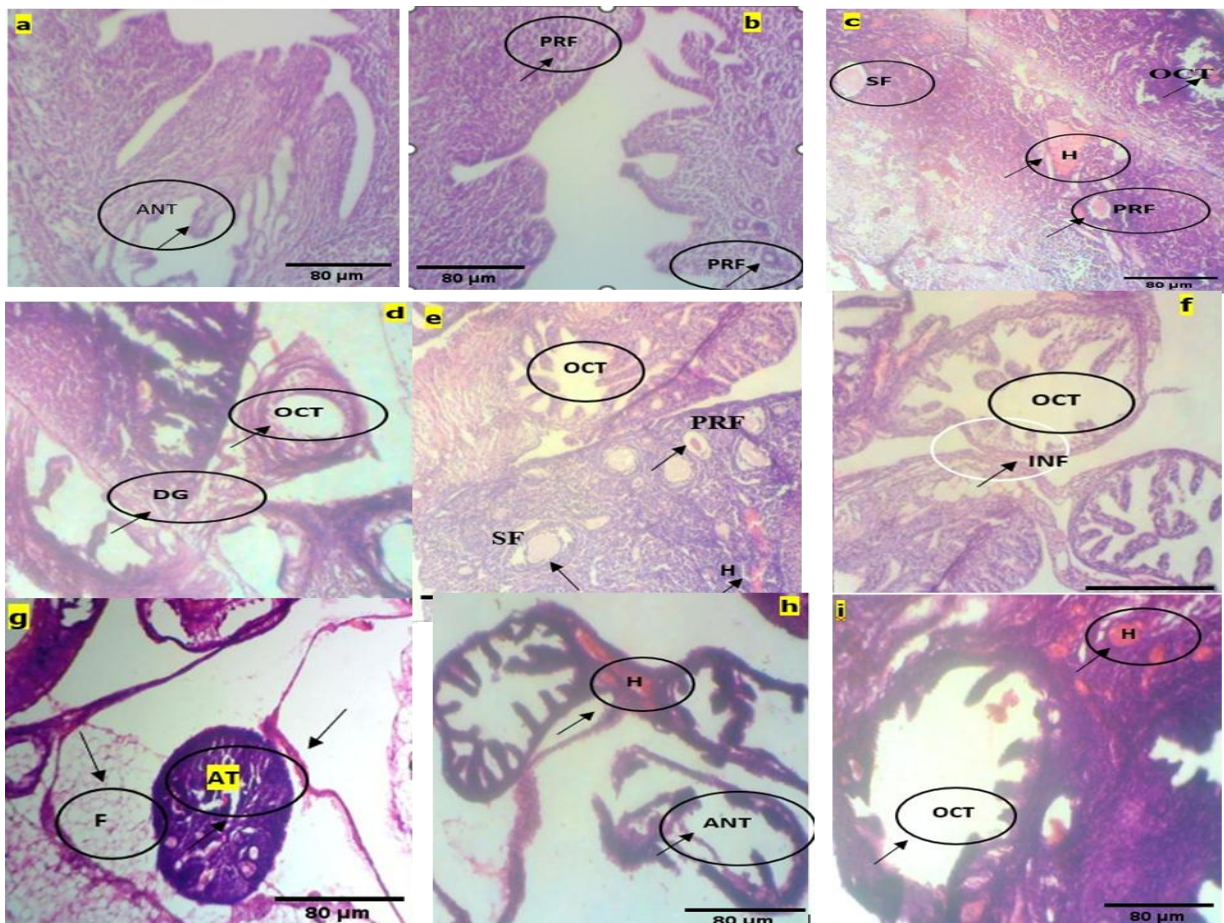


Plate 1. Photomicrograph showing the ovary of mice treated with ACT-tenderized for the period of this study. 1a and 21 shows the normal ovary with no pathological evidence with the antrum (ANT) and primordial follicles (PRF), (c) presence of haemorrhage (H) on day 15 (d) degeneration of the zona pellucidum (DG) on day 15 (e) presence of haemorrhage (H) on day 25 (f) inflammations (INF) around the oocyte on day 25 (H), (g) Atrophy change at the oocyte (AT) on day 35(h) haemorrhage (H) in ovarian tissue on day 35, (i) severe incidence of haemorrhage on day 35.

4. Discussion

This study investigated the reproductive toxicity of ACT-tenderized meat in female Swiss mice, with particular emphasis on key reproductive biomarkers such as estradiol, luteinizing hormone (LH), follicle-stimulating hormone (FSH), and progesterone, and on histopathological alterations in the ovaries. These biomarkers were selected due to their central roles in regulating the estrous cycle and fertility. Estradiol is critical for follicular development and ovulation (Xu et al., 2022); LH initiates ovulation via its surge following the estrogen peak (Al-Fahham and Al-Nowainy, 2016); FSH stimulates follicle maturation (Plant et al., 2024); while progesterone prepares and maintains the uterus for pregnancy (Sophia, 2003). In this study, the marked elevation in estradiol in Groups 2 and 3 suggests disrupted estrogen regulation after ACT tenderized meat exposure. Prior mouse data indicate that ACT can delay estradiol, LH, and FSH production and reduce proestrus frequency, so the increase here may reflect altered estrous-cycle dynamics rather than sustained estradiol overproduction (Ndeke, 2022). While HPG-axis feedback disruption is a probable mechanism, this study does not directly demonstrate it. Given the essential role of estradiol in normal ovulation, this hormonal disturbance may have implications for fertility. (McIlwraith & Belsham, 2019)."

The nonsignificant increase in LH observed in Group 4 may be a reflection of an altered preovulatory endocrine signaling, due to the fact that the elevation of estradiol normally drives positive feedback on the hypothalamic-pituitary axis to generate the LH surge (Kauffman, 2022). This interpretation is consistent with evidence that ACT exposure in female mice delays LH, FSH, and estradiol production and reduces proestrus frequency. Thus, suggesting a disruption of hormonal synchrony rather than a stable shift in baseline gonadotropin levels (Ndeke, 2022). The non-significant increase in progesterone levels in this study contrasted with earlier studies, which reported that ACT may reduce progesterone production during certain estrous stages (Wall et al., 2023). This discrepancy may be explained by differences in exposure route. Previous studies involved controlled pharmacological dosing, whereas the present study mimicked dietary exposure through adulterated meat. The rise observed here may therefore represent disrupted cycle timing rather than consistent enhancement, reinforcing the notion that ACT primarily affects hormonal rhythms rather than absolute secretion.

The marked fall in ovarian weight across all treated groups indicates substantial loss of ovarian mass, which usually reflects depletion of follicles, stromal remodeling, or both (Bazzano et al., 2023; Zeng et al., 2023). This interpretation is consistent with prenatal ACT mouse data showing decreased ovarian weight and impaired cyclicity in offspring (Bazzano et al., 2023) It also aligns with the study of Arendrup et al. (2017), they reported incidences of significantly lower ovary weight after developmental ACT exposure, together with fewer corpora lutea and more cyst-like ovarian structures later in life. A reduction in ovarian weight is plausible because ACT has been linked to reduced cell proliferation, increased cell death, and impaired steroidogenic support in ovarian tissue (Lecante et al., 2022). In a study that involved the use of fetal human ovary explants, ACT was observed to cause a decrease in total cell number, reduced KI67-positive proliferating cells, and increased apoptosis and necrosis, especially in the more sensitive 10- to 12-week developmental window (Lecante et al., 2022). Furthermore, mouse fetal exposure studies similarly found reduced oocyte number, inhibited cell

proliferation, and suppression of markers of follicular development and pre-granulosa steroidogenic function (Wu et al., 2024).

The histological evaluation of ovarian tissue in mice fed with ACT tenderized meat revealed a clear, time-dependent progression of pathological alterations. As the duration of exposure increased from 15 to 35 days, the severity of tissue damage increased from early degenerative changes to more intense structural abnormalities. This chronological escalation indicates that potential thermal degradation products of ACT like 4-aminophenol, hydroquinone, p-nitrophenol, and NAPQI (Ezugwu et al., 2023), exerted a cumulative and progressive toxic effects on the female reproductive system, mirroring standard toxicological frameworks for classifying microscopic lesions observed in the female reproductive tract of laboratory rats and mice (Dixon et al., 2014). Granulosa cells play a vital role in providing nutritional support, maintaining gap junction communication, and synthesizing steroid hormones necessary for oocyte maturation (Sen Halicioglu et al., 2021). The early degeneration of these cells after 15 days suggests that the toxic agents in the treated mice rapidly targeted the follicular microenvironment, potentially disrupting normal folliculogenesis and hormone production early in the exposure cycle (Berkel, 2024). The persistent presence of haemorrhage coupled with the emergence of inflammation (INF) surrounding the oocyte after 25 days of exposure suggests an active inflammatory response. In ovarian toxicology, such inflammation is frequently driven by oxidative stress cascades and pro-inflammatory signaling pathways (such as NLRP3 inflammasome activation), which can trigger pyroptotic or apoptotic death in surrounding granulosa cells (Berkel, 2024). Prolonged inflammation within the ovarian stroma is highly detrimental, it alters the vascular architecture and creates a toxic microenvironment that compromises oocyte viability accelerates follicular atresia. The presence of atrophy (AT) in the primary oocyte on day 35 highlights a severe threat to reproductive longevity, suggesting that prolonged exposure induces direct oocyte depletion, loss of developmental competence, or speed up ovarian aging (Contestabile, 2026). Furthermore, the development of fibrosis marks a transition from acute, reversible injury to chronic, irreversible tissue remodeling. In the ovary, chronic or repetitive injury triggers excessive extracellular matrix (ECM) deposition by activated myofibroblasts, typically driven by key fibrotic signaling pathways like TGF- β /Smad (Edepli, 2026). This fibrotic replacement of functional ovarian parenchyma with scar tissue may stiffen the organ and disrupts its complex architectural network. Thus, culminating in severe ovarian dysfunction or premature ovarian failure.

Conclusion

This study revealed that the consumption of ACT-tenderized meat may negatively affect female reproductive function in Swiss mice, as shown by altered reproductive hormone levels, reduced ovarian weight, and progressive ovarian tissue damage. The hormonal changes observed suggest disruption of endocrine regulation and estrous cycle coordination, which may impair normal follicular development and ovulation. The reduction in ovarian weight and histological findings of granulosa cell degeneration, hemorrhage, inflammation, oocyte atrophy, and fibrosis indicate progressive ovarian injury with prolonged exposure. These changes suggest that ACT-derived compounds may contribute to oxidative stress, inflammation, and structural damage within ovarian tissue, potentially compromising fertility.

Overall, the findings highlight possible reproductive risks associated with dietary exposure to ACT-contaminated meat and emphasize the need for further studies to clarify the underlying mechanisms and long-term effects.

Declarations

Ethical Clearance

Before the commencement of the study, an Ethical approval was granted by the Centre for Research and Development (CERAD), Federal University of Technology. The approval number was FUTA/ETH/25/238. All protocol and guidelines regarding animal welfare and handling were strictly adhered to throughout the study period.

Conflict of Interest

The authors declare that they have no financial or personal interests that could have affected the conduct or findings of this study.

Funding

This study received no specific funding from public, commercial, or not-for-profit funding organizations.

Use of generative AI and AI-assisted technologies

The author(s) used ChatGPT (OpenAI) to support language editing. All AI-assisted content was subsequently reviewed and revised by the author(s), who take full responsibility for the final manuscript.

References

- Al-Fahham, A. A., & Al-Nowainy, H. Q. (2016). The role of FSH, LH, and prolactin hormones in female infertility. *American Journal of PharmTech Research*, 6(5), 110–118.
- Arendrup, F. S., Mazaud-Guittot, S., Jégou, B., & Kristensen, D. (2017). EDC IMPACT: Is exposure during pregnancy to acetaminophen/paracetamol disrupting female reproductive development? *Endocrine Connections*, 7, 149–158. <https://doi.org/10.1530/EC-17-0298>
- Banihani, S. A. (2018). Effect of paracetamol on semen quality. *Andrologia*, 50(1). <https://doi.org/10.1111/and.12874>
- Bazzano, M. V., Berkout, L., Bremmer, L., Weber, F., Hardardottir, L., Promm, M., Königer, A., Arck, P. C., Bosurgi, L., Tiegs, G., & Solano, M. E. (2023). Prenatal acetaminophen exposure distorts ovarian macrophages with long-lasting consequences for gonadal function. *Journal of Reproductive Immunology*, 158, Article 103565. <https://doi.org/10.1016/j.jri.2022.103565>
- Bekhit, A. A., Hopkins, D. L., Geesink, G., Bekhit, A. A., & Franks, P. (2014). Exogenous proteases for meat tenderization. *Critical Reviews in Food Science and Nutrition*, 54(8), 1012–1031.

- Berkel, C. (2024). Inducers and inhibitors of pyroptotic death of granulosa cells in models of premature ovarian insufficiency and polycystic ovary syndrome. *Reproductive Sciences*, 31(10), 2972–2992. <https://doi.org/10.1007/s43032-024-01643-3>
- Chen, Z., Sun, X., Liu, Y., Zhao, X., Guo, Y., & Wang, H. (2024). The characterization of developmental toxicity in fetal offspring induced by acetaminophen exposure during pregnancy. *Ecotoxicology and Environmental Safety*, 283, Article 116980. <https://doi.org/10.1016/j.ecoenv.2024.116980>
- Contestabile, M. (2026). Endometriosis and oocyte quality: Morphological alterations, developmental competence, and modifiable strategies for reproductive longevity. *Cells*, 15(3), 296. <https://doi.org/10.3390/cells15030296>
- Dixon, D., Alison, R., Bach, U., Colman, K., Foley, G. L., Harleman, J. H., Haworth, R., Herbert, R., Heuser, A., Long, G., Mirsky, M., Regan, K., Van Esch, E., Westwood, F. R., Vidal, J., & Yoshida, M. (2014). Nonproliferative and proliferative lesions of the rat and mouse female reproductive system. *Journal of Toxicologic Pathology*, 27(1 Suppl.), 1S–107S. <https://doi.org/10.1293/tox.27.1s>
- Edepli, B. G. (2026). Molecular mechanisms of ovarian fibrosis. *Journal of Ovarian Research*, 19(1), 12776365.
- European Centre for Ecotoxicology and Toxicology of Chemicals. (2002). *Guidance on evaluation of reproductive toxicity data* (ECETOC Monograph No. 31). ECETOC.
- Ezugwu, A. L., Anaduaka, E. G., Chibuogwu, C. C., & Ezeorba, T. P. C. (2023). Meat tenderization using acetaminophen (paracetamol/APAP): A review on deductive biochemical mechanisms, toxicological implications and strategies for mitigation. *Heliyon*, 9(5), e15628. <https://doi.org/10.1016/j.heliyon.2023.e15628>
- Hashem, S. M., Al-Rekabi, F. M. K., & Ali, S. (2023). Evaluation of the acute toxicity of acetaminophen in mice. *Iraqi Journal of Veterinary Medicine*, 47(2), 81–87.
- Kadhim, H. M. (2024). Impact of anti-Müllerian hormone, oxidative stress and lipid profile levels on female fertility. *Medical Science Journal of Advanced Research*, 5, 1–8. <https://doi.org/10.46966/msjar.v5i4.244>
- Kauffman, A. S. (2022). Neuroendocrine mechanisms underlying estrogen positive feedback and the LH surge. *Frontiers in Neuroscience*, 16, 953252. <https://doi.org/10.3389/fnins.2022.953252>
- Lecante, L., Leverrier-Penna, S., Gicquel, T., Giton, F., Costet, N., Desdoits-Lethimonier, C., Lesné, L., Fromenty, B., Lavoué, V., Rolland, A., & Mazaud-Guittot, S. (2022). Acetaminophen (APAP, paracetamol) interferes with the first trimester human fetal ovary development in an ex vivo model. *The Journal of Clinical Endocrinology & Metabolism*, 107, 1647–1661. <https://doi.org/10.1210/clinem/dgac080>
- Lee, C. Y., Hwang, H., Park, J. S., Lee, S. H., Park, C. E., Cheon, Y. P., & Choi, D. (2023). Effects of acetaminophen on reproductive activities in male golden hamsters.

- Development & Reproduction*, 27(1), 25–37.
<https://doi.org/10.12717/DR.2023.27.1.25>
- Madhusankha, G. D. M. P., & Thilakarathna, R. C. N. (2020). Meat tenderization mechanism and the impact of plant exogenous proteases: A review. *Arabian Journal of Chemistry*. <https://doi.org/10.1016/j.arabjc.2020.102967>
- Marques, P., De Sousa Lages, A., Skorupskaite, K., et al. (2025). Physiology of GnRH and gonadotrophin secretion. In K. R. Feingold et al. (Eds.), *Endotext*. MDText.com. <https://www.ncbi.nlm.nih.gov/books/NBK279070/>
- McIlwraith, E., & Belsham, D. (2019). Analysis of palmitate- and bisphenol A-mediated changes in microRNAs targeting the novel reproductive peptide phoenixin and its receptor Gpr173 in hypothalamic cell lines. *Journal of the Endocrine Society*, 3(Suppl. 1), SUN-475. <https://doi.org/10.1210/js.2019-SUN-475>
- Mohd Azmi, S. I., Kumar, P., Sharma, N., Sazili, A. Q., Lee, S.-J., & Ismail-Fitry, M. R. (2023). Application of plant proteases in meat tenderization: Recent trends and future prospects. *Foods*, 12(6), 1336. <https://doi.org/10.3390/foods12061336>
- Moles, A., Torres, S., Baulies, A., Garcia-Ruiz, C., & Fernandez-Checa, J. C. (2018). Mitochondrial-lysosomal axis in acetaminophen hepatotoxicity. *Frontiers in Pharmacology*, 9, 453. <https://doi.org/10.3389/fphar.2018.00453>
- Nielsen, B. S., Petersen, M. R., Martin-Gonzalez, J., Holmberg, C., Mjoseng, H. K., Frederiksen, H., et al. (2025). Paracetamol (N-acetyl-para-aminophenol) disrupts early embryogenesis by cell cycle inhibition. *Human Reproduction*, 40(10), 1860–1876. <https://doi.org/10.1093/humrep/deaf116>
- Okuthe, G. E., Dube, E., & Mafunda, P. S. (2025). Effects of pharmaceuticals and endocrine-disrupting chemicals on reproductive biology of aquatic fauna: Penguins as sentinel species. *Journal of Xenobiotics*, 15(4), 110. <https://doi.org/10.3390/jox15040110>
- Perlman, R. L. (2016). Mouse models of human disease: An evolutionary perspective. *Evolution, Medicine, and Public Health*, 2016(1), 170–176. <https://doi.org/10.1093/emph/eow014>
- Reel, J. R., Lawton, A. D., & Lamb, J. C., IV. (1992). Reproductive toxicity evaluation of acetaminophen in Swiss CD-1 mice using a continuous breeding protocol. *Fundamental and Applied Toxicology*, 18(2), 233–239. [https://doi.org/10.1016/0272-0590\(92\)90051-I](https://doi.org/10.1016/0272-0590(92)90051-I)
- Rosner, J., Samardzic, T., & Sarao, M. S. (2025). Physiology, female reproduction. In *StatPearls*. StatPearls Publishing. <https://www.ncbi.nlm.nih.gov/books/NBK537132/>
- Sen Halicioglu, B., Saadat, K. A. S. M., & Tuglu, M. I. (2021). The relationship of 4-vinylcyclohexene diepoxide toxicity with cell death, oxidative stress, and gap junctions in female rat ovaries. *Reproductive Medicine and Biology*, 20(4), 543–553. <https://doi.org/10.1002/rmb2.12398>

- Shi, H., Shahidi, F., Wang, J., Huang, Y., Zou, Y., Xu, W., & Wang, D. (2021). Techniques for postmortem tenderisation in meat processing: Effectiveness, application and possible mechanisms. *Food Production, Processing and Nutrition*, 3, 1–26. <https://doi.org/10.1186/S43014-021-00062-0>
- Sophia, T. (2003). Female reproductive endocrinology. *Journal of Reproductive Biology*, 45(2), 89–95.
- Tricotteaux-Zarqaoui, S., Lahimer, M., Abou Diwan, M., Corona, A., Candela, P., Cabry, R., Bach, V., Khorsi-Cauet, H., & Benkhalifa, M. (2024). Endocrine disruptor chemicals exposure and female fertility declining: From pathophysiology to epigenetic risks. *Frontiers in Public Health*, 12, 1466967. <https://doi.org/10.3389/fpubh.2024.1466967>
- Williams, C. J., & Erickson, G. F. (2012). Morphology and physiology of the ovary. In K. R. Feingold et al. (Eds.), *Endotext*. MDText.com. <https://www.ncbi.nlm.nih.gov/books/NBK278951/>
- Wu, T., Huang, J., Li, Y., Guo, Y., Wang, H., & Zhang, Y. (2024). Prenatal acetaminophen exposure and the developing ovary: Time-, dose-, and course-dependent consequences for fetal mice. *Food and Chemical Toxicology*. <https://doi.org/10.1016/j.fct.2024.114679>
- Wu, X., Tian, Y., Zhu, H., Xu, P., Zhang, J., Hu, Y., Ji, X., Yan, R., Yue, H., & Sang, N. (2023). Invisible hand behind female reproductive disorders: Bisphenols, recent evidence and future perspectives. *Toxics*, 11(12), 1000. <https://doi.org/10.3390/toxics11121000>
- Xu, X.-L., Huang, Z.-Y., Yu, K., Li, J., Fu, X.-W., & Deng, S.-L. (2022). Estrogen biosynthesis and signal transduction in ovarian disease. *Frontiers in Endocrinology*, 13, Article 827032.
- Zeng, L., Zhou, C., Xu, W., Huang, Y., Wang, W., Ma, Z., Huang, J., Li, J., Hu, L., Xue, Y., Luo, T., & Zheng, L. (2023). The ovarian-related effects of polystyrene nanoplastics on human ovarian granulosa cells and female mice. *Ecotoxicology and Environmental Safety*, 257, Article 114941. <https://doi.org/10.1016/j.ecoenv.2023.114941>