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Potential Effects of Tempeh Extract on Ovarian Folliculogenesis in Female Wistar Rats

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ABSTRACT
Phytoestrogens such as genistein, abundantly found in tempeh—a traditional Indonesian fermented soybean product—have been shown to exhibit estrogen-like activities. These compounds may influence ovarian function, particularly folliculogenesis, but their dose-dependent effects remain controversial. This study aimed to investigate the potential effects of tempeh extract on ovarian folliculogenesis in female Wistar rats as an experimental model to assess the possible reproductive implications of phytoestrogen consumption. This true experimental study used a randomized post-test only control group design and was conducted as part of the first author's undergraduate thesis at Universitas Brawijaya. Twenty-four healthy female Wistar rats (aged 4–8 weeks) were divided into four groups (n = 6). The control group received no treatment, while treatment groups received oral tempeh extract at doses of 7.5 mg, 15 mg, and 30 mg per 200 g body weight for 28 days. On day 29, ovarian tissues were collected, stained, and examined histologically to quantify primary, secondary, Graafian, and atretic follicles. Data were analyzed using non-parametric statistical tests. Although no statistically significant differences were observed among groups ($p > 0.05$), a descriptive trend indicated a reduction in early-stage follicles and an increase in Graafian and atretic follicles in the treatment groups. These changes suggest dose-dependent modulation of folliculogenesis. Tempeh extract may influence ovarian follicular dynamics, potentially accelerating follicular maturation or atresia. These findings highlight the need for further research to evaluate the reproductive safety of long-term phytoestrogen exposure from soy-based diets in women of reproductive age.

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1. INTRODUCTION

The female reproductive system consists of specialized organs that work in coordination to ensure successful reproduction. Among these, the ovaries and uterus play pivotal roles. The uterus serves as the site for embryo implantation and fetal development, while the ovaries are responsible for producing oocytes and key reproductive hormones, including estrogen, progesterone, inhibin, and testosterone. These hormones regulate the menstrual cycle and maintain reproductive homeostasis. Estrogen is essential for the maturation and function of reproductive organs such as the ovaries and fallopian tubes. As puberty begins, increased secretion of gonadotropin-releasing hormone (GnRH) from the hypothalamus stimulates the anterior pituitary to release luteinizing hormone (LH) and follicle-stimulating hormone (FSH), both of which promote follicular development and estrogen synthesis. Estrogens are primarily produced in the ovaries through the aromatization

of androgens by the enzyme aromatase (p450 aromatase), although extragonadal estrogen production can also occur in responsive tissues (1).

Maintaining adequate estrogen levels is critical for sustaining normal ovarian function and overall female reproductive health. One condition closely associated with estrogen deficiency is premature ovarian insufficiency (POI), characterized by the early depletion of ovarian follicles and a decline in fertility before the age of 40. Recent studies have highlighted oxidative stress as a central factor contributing to ovarian aging and dysfunction in POI, with mitochondrial damage, apoptosis, and chronic inflammation playing key roles in follicular atresia. Contributing factors to increased oxidative stress include genetic mutations, autoimmune disorders, chemotherapeutic agents, and lifestyle-related aspects such as poor diet, low body mass index (BMI), and inadequate energy intake. These findings suggest that dietary interventions may offer protective effects against POI by modulating oxidative stress and preserving hormonal balance. Therefore, identifying and evaluating foods with potential estrogenic or antioxidant properties, such as fermented soy-based products, is essential for reducing the risk of early ovarian failure and improving reproductive longevity (2).

Building on the crucial role of estrogen in regulating ovarian function, increasing attention has been directed toward compounds that can mimic or influence its activity. One such compound is genistein, a phytoestrogen abundantly found in tempeh—a traditional fermented soybean product widely consumed in Indonesia. Phytoestrogens are plant-derived molecules that resemble endogenous estrogens in structure and are capable of binding to estrogen receptors within the body. Genistein, in particular, shows a stronger affinity for estrogen receptor beta (ERβ) than for estrogen receptor alpha (ERα), allowing it to exert estrogen-like or modulatory effects depending on the physiological context. In many Asian populations, where soy-based foods such as tempeh, tofu, and soy milk form a significant part of the diet, genistein serves as a primary dietary source of phytoestrogens. Considering the role of estrogen in the growth and maturation of ovarian follicles, genistein may have the potential to influence these reproductive processes. Studies have indicated that genistein can impact the number and development of follicles, regulate levels of steroid hormones, and affect the expression of genes involved in cell cycle control and steroid biosynthesis. Interestingly, these effects tend to be dose-dependent, with low doses often promoting cell proliferation, while higher concentrations may inhibit it (3).

Phytoestrogens have also been associated with potential health benefits, including the alleviation of menopausal symptoms—such as hot flashes—when consumed directly through phytoestrogen-rich foods or supplements. Moreover, they have been linked to enhanced bone formation in postmenopausal women and cardiovascular protection, particularly through reductions in plasma cholesterol that may delay atherosclerosis and improve vascular function. However, due to their dualistic capacity to act as estrogen agonists or antagonists, phytoestrogens are classified as endocrine-disrupting chemicals (EDCs). EDCs are known to interfere with hormonal signaling and have the potential to affect the development of the nervous, immune, and reproductive systems. The reproductive-disrupting potential of phytoestrogens has been reported in livestock such as sheep and cattle, which experienced infertility after consuming estrogen-rich plants. Human studies have also noted adverse effects, such as an increased risk of early menarche among individuals who consumed soy-based products heavily during infancy, possibly due to early exposure to estrogen-like soy isoflavones (4). Other studies have also shown that phytoestrogens can negatively affect women's reproductive health, including interfering with the development of reproductive organs, prolonging the estrous cycle, causing fluid accumulation in the uterus, inhibiting ovulation, and increasing the risk of infertility. These effects are associated with phytoestrogen exposure in the dose range of 0.5 to 291 mg/kg/day. The types of phytoestrogens known to contribute to these disorders include the isoflavone, coumestane, flavanone, and flavone groups. The effect of phytoestrogens on reproductive organs such as the uterus, ovaries, fallopian tubes and vagina is strongly influenced by the dose and duration of exposure (4).

While numerous studies have explored isolated isoflavones such as genistein, fewer have focused on the effects of tempeh as a whole food matrix, where fermentation and other bioactive compounds may interact and influence biological responses differently. Genistein, a principal isoflavone found in tempeh, has been extensively researched for its various pharmacological attributes. Preclinical studies have evidenced its antioxidant, anti-inflammatory, anti-cancer, and estrogenic properties, mostly due to its structural resemblance to natural estrogen, enabling it to competitively bind to estrogen receptors. Nonetheless, despite its possible therapeutic advantages, genistein may displace endogenous estrogen and disrupt hormonal equilibrium at specific dosages (5). Sharifi-Rad et al. (2021) conducted a thorough study highlighting the necessity for

additional research on its long-term efficacy, safety, and bioavailability in humans. In the other of animal study, the administration of soy isoflavones to female Wistar rats at doses ranging from 50 to 200 mg/kg/day from weaning to sexual maturity resulted in decreased serum estradiol levels and an elevation in ovarian follicle atresia (6). The findings indicate that extended exposure to modest levels of soy-derived phytoestrogens may cause metabolic disruptions in follicular fluid and potentially hinder folliculogenesis. Consequently, assessing the impact of naturally fermented soy products such as tempeh, which possess a complex array of isoflavones and fermentation byproducts, is crucial for comprehending their protective and detrimental roles in reproductive physiology.

Based on this background, it is important to explore the effect of natural phytoestrogens such as those found in tempeh on the reproductive system, especially in the context of the number of ovarian follicles. This study aims to determine the effect of tempeh extract administration on the number of ovarian follicles in female Wistar strain white rats (*Rattus norvegicus*) as an experimental model to understand the potential benefits or risks of phytoestrogens on ovarian function.

2. METHOD

This study was conducted as part of the first author's undergraduate thesis at the Midwifery Department, Universitas Brawijaya. Although the experimental work was completed in 2017, the data remain scientifically relevant due to the true experimental design, strict adherence to standard laboratory procedures, and the biological consistency of the study parameters. The findings have not been previously published and are presented here for the first time.

A true experimental design with a randomized post-test only control group approach was employed. Twenty-four healthy female Wistar rats (*Rattus norvegicus*), aged 4 to 8 weeks, were randomly assigned into four groups (n = 6 per group). Group I served as the control and received no treatment. Groups II, III, and IV were treated with tempeh extract at doses of 7.5 mg, 15 mg, and 30 mg per 200 g body weight per day, respectively.

Tempeh extract was administered orally once daily for 28 consecutive days using an orogastric tube. The extract was diluted in distilled water to ensure accurate and consistent dosing. On day 29, all animals were euthanized during the estrus phase, and their ovaries were surgically removed following standardized protocols.

Ovarian tissues were fixed, processed for histological examination, and stained with hematoxylin and eosin. Follicular structures—including primary, secondary, Graafian, and atretic follicles—were examined and counted under a light microscope at 400× magnification. All experimental procedures were approved by the Ethics Committee of Universitas Brawijaya (Ethical clearance no. 304/EC/KEPK-S1-KB/06/2017).

Data were analyzed using SPSS version 16.0. The Kruskal–Wallis test was used to assess differences among groups, followed by Mann–Whitney U tests for post hoc comparisons. Spearman's rank correlation coefficient was used to evaluate associations between variables. Randomization and consistency in experimental procedures ensured the reliability and validity of the study results.

3. RESULTS AND DISCUSSION

3.1. Ovarian Follicle Profile and Statistical Analysis

Figure 1 presents the average number (± SD) of ovarian follicle types—primary, secondary, Graafian, and atretic—across the four experimental groups. The control group (0 mg/200 g BW) exhibited the highest mean counts of early-stage follicles, including primary follicles (19.00 ± 7.71) and secondary follicles (2.64 ± 2.42). In contrast, the treatment groups demonstrated lower counts of these early-stage follicles, with the lowest primary follicle count observed in T2 (15.00 ± 4.93) and the lowest secondary follicle count in T3 (1.89 ± 0.93).

Conversely, the number of Graafian follicles increased in response to tempeh extract treatment, peaking in T2 (0.50 ± 0.53) compared to the control group (0.09 ± 0.30). A similar trend was observed in atretic follicles, where T2 also showed the highest count (8.90 ± 5.55), while the control group had the lowest (5.91 ± 3.15). These observations suggest a potential shift from early-stage to more advanced or degenerating follicular stages following extract administration.

Despite these descriptive differences, statistical analysis using the Kruskal–Wallis test revealed no significant differences among the groups for any follicle type (p-values for primary, secondary, Graafian, and atretic follicles were 0.489; 0.894; 0.206; and 0.474, respectively; $p > 0.05$). Nonetheless, the biological trend

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points toward a dose-related modulation of folliculogenesis, with potential implications for ovarian development.

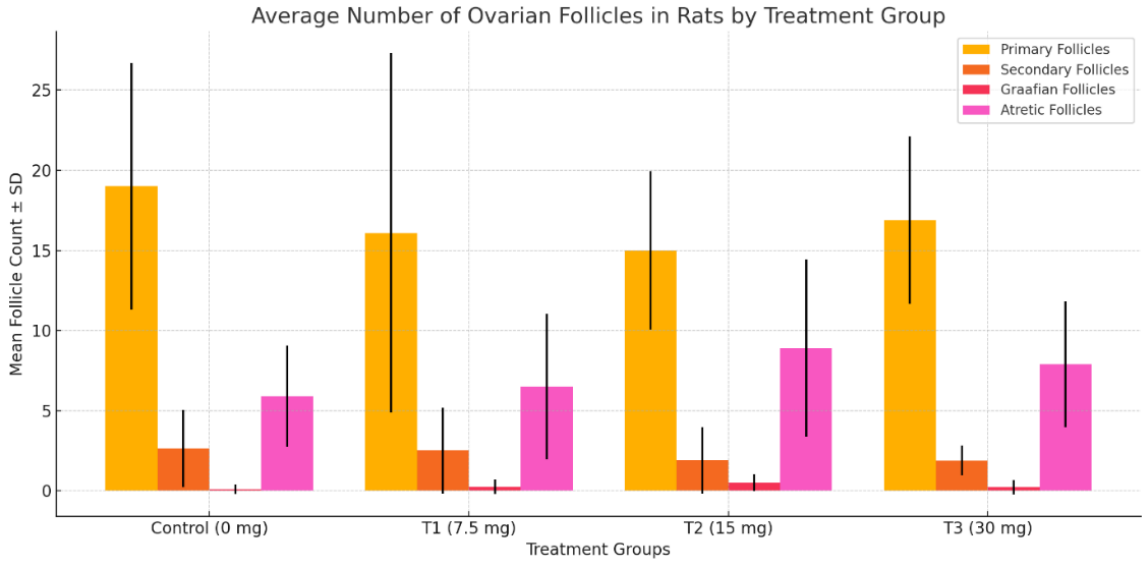

Figure 1. Average Number of Ovarian Follicles in Rats by Treatment Group (Mean ± SD)
Primary, secondary, Graafian, and atretic follicles were quantified histologically from ovarian tissue samples collected after 28 days of treatment. Each group consisted of six female Wistar rats (n = 6). Follicle counts are presented as means with standard deviations (error bars). No statistically significant differences were found among groups ($p > 0.05$, Kruskal–Wallis test).

3.2. Possible Role of Isoflavones in Follicular Changes

Isoflavones are a group of phenolic compounds that include phytoestrogens because they have a chemical structure similar to estrogen. Isoflavones are found in plants from the Fabaceae family, and the main source in the diet comes from soybeans and their processed products, such as tempeh. These compounds are known to have various benefits for human health, such as reducing the risk of cardiovascular disease, improving quality of life during menopause, reducing the risk of several types of cancer, and supporting nervous system health, especially in old age(7).

However, in nature, isoflavones generally exist in bound forms, such as glycosylates and methylates, which cannot be directly absorbed by the gut. In order to be absorbed and show higher biological activity, these forms need to be metabolized first by the gut microbiota. Therefore, the effectiveness of isoflavones in providing health benefits largely depends on the composition of one's gut microbiota. The presence of certain types of bacteria plays an important role in converting isoflavones into active forms such as genistein, equol, and O-desmethylangolensin (O-DMA), which have different biological effects compared to their initial forms (7).

This shift may be attributed to the isoflavone content in tempeh, particularly genistein. Genistein is known to modulate hormone levels and has shown beneficial effects in managing hormonal imbalances, such as in PCOS (8). However, its effects are dose-dependent and context-specific. A previous study reported that neonatal rats exposed to genistein (0.5–50 mg/kg) experienced impaired ovarian development and reproductive dysfunction, including infertility at high doses (9). The observed decrease in primary and secondary follicles and concurrent increase in atretic follicles may be attributed to the phytoestrogen content of tempeh, particularly genistein. These findings support previous evidence that genistein can influence ovarian folliculogenesis in a dose-dependent and context-specific manner. While moderate doses may enhance maturation, excessive or prolonged exposure can disrupt estrogen signaling and induce follicular atresia.

3.3. Mechanism of Genistein Action

The metabolic processing of genistein, a prominent isoflavone found in soy-based foods, primarily occurs in the gastrointestinal tract. During intestinal absorption, genistein undergoes conjugation with

glucuronic acid. A portion of this compound is subsequently transported to the liver, where it is secreted into the bile and returns to the small intestine, enabling enterohepatic recirculation and further metabolic transformation. Enzymes such as β-glucosidase and phenol sulfatase play key roles in converting genistein into its biologically active, lipophilic aglycone form, which can be readily absorbed or further metabolized by intestinal microbiota.

Certain gut bacteria, including Escherichia coli strain HGH21 and the Gram-positive strain HGH6, have been identified to convert genistein into 5-hydroxymarinol derivatives. These metabolites exhibit notable biological activity, including inhibition of proliferation, migration, and invasion in human liver cancer cell lines such as SMMC-7721 and HepG2 in in vitro studies.

As a phytoestrogen, genistein can bind to both estrogen receptor subtypes—ERα and ERβ—thereby influencing hormonal signaling pathways. In males, it may interfere with testosterone synthesis, potentially contributing to hormonal imbalance and impaired testicular function. Evidence from dietary intervention studies suggests that intake of soy protein containing genistein at doses around 56 grams per day over four weeks can result in a significant decline (approximately 19%) in serum testosterone levels. In females, especially at higher exposures, genistein may affect ovarian function through multiple mechanisms, exhibiting both estrogen-like actions and inhibitory effects on steroidogenesis, potentially leading to reduced ovarian activity (10).

Another study explained that the administration of genistein in various doses showed a decrease in estrogen receptor (ER) expression (11). These physiological effects of genistein are largely attributed to its structural resemblance to estradiol (E2), the primary form of estrogen in humans. In its aglycone form, genistein exhibits the ability to interact with estrogen receptors—both ERα and ERβ—thereby mimicking or modulating endogenous estrogen activity. Depending on the surrounding hormonal milieu, genistein may act as either an estrogen receptor agonist or antagonist. This dual functionality allows genistein to exert context-dependent effects on reproductive tissues and endocrine regulation, further emphasizing its complex role as a dietary phytoestrogen with potential therapeutic and disruptive properties (5). This dual role allows genistein to stimulate or inhibit estrogenic responses, thereby influencing ovarian follicle dynamics.

3.4. Study Limitations and Future Directions

The lack of statistical significance may reflect biological variation, limited sample sizes, or differences between human and animal models. Larger-scale studies with varied dosages are needed to draw definitive conclusions. Moreover, although this study was performed in animals, it provides an early indication of how phytoestrogens from tempeh might affect reproductive health.

3.5. Implications for Human Health

Tempeh is a traditional fermented product originating from Indonesia. Indonesians have been consuming tempeh for a long time as an affordable and cheap source of protein. Tempeh is generally made from soybeans that undergo fermentation by Rhizopus spp (12). Given that tempeh is a widely consumed soy-based food in several cultures, understanding its impact is vital. Further clinical research involving women of reproductive age is necessary to determine both the safety and potential reproductive health benefits of long-term tempeh consumption. Considering the widespread consumption of tempeh in Indonesia and other cultures, this study highlights the importance of monitoring phytoestrogen intake in women of reproductive age. Although tempeh is a nutritious and affordable protein source, its potential endocrine-modulating effects warrant further investigation in clinical settings to inform dietary guidelines and reproductive health policy.

4. CONCLUSION

This study demonstrates a biological inclination that the administration of tempeh extract may influence ovarian folliculogenesis in female Wistar rats, evidenced by a potential reduction in the number of primary follicles and an increase in de Graaf and atretic follicles, albeit not statistically significant. The results suggest that phytoestrogens in tempeh, particularly genistein, can influence follicle development in a dose-dependent fashion.

Considering that tempeh is a staple in the diets of Indonesians, particularly among women of reproductive age, our findings underscore the necessity for additional study on the effects of prolonged phytoestrogen intake on reproductive health.

Healthcare professionals should contemplate the possible reproductive consequences of elevated phytoestrogen intake, particularly when educating patients with hormonal abnormalities or fertility issues. Educational institutions, particularly in obstetrics and health sciences, should incorporate evidence-based knowledge regarding phytoestrogens into the curriculum to enhance nutritional education and promote public health. Additional clinical investigations are required to investigate the safe ingestion threshold and individual sensitivity to phytoestrogens.

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