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RESEARCH ARTICLE

Effects of isoflavones soybean extract on luteinizing hormone, follicle-stimulating hormone levels, and ovarian antral follicle diameter in female mice

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ABSTRACT

Hormonal contraceptives can cause side effects, prompting interest in natural compounds such as soybean isoflavones, which may interact with estrogen receptors and influence reproductive hormones. This study evaluated the effects of soybean extract isoflavones on luteinizing hormone (LH), follicle-stimulating hormone (FSH), and ovarian antral follicle diameter in female mice. Fifteen healthy mice were randomised into three groups (n = 5 each): control (aquadest), P1 (4.5 mg isoflavone per dose), and P2 (9 mg isoflavone per dose). Treatments were administered orally twice daily for 15 days. LH and FSH were measured by ELISA, and antral follicle diameter was assessed histologically. The Kruskal–Wallis's test showed no significant differences among groups for LH ($H = 1.982$, $p = 0.371$), FSH ($H = 1.123$, $p = 0.570$), or antral follicle diameter ($H = 1.220$, $p = 0.543$). Although descriptive variations were observed, they were not statistically significant. These results suggest that 15-day oral administration of soybean extract isoflavones at the tested doses does not significantly alter LH, FSH, or ovarian antral follicle diameter in healthy female mice; larger, longer, and better-controlled studies are recommended.

1. Introduction

Family planning enables individuals and couples to determine the number and spacing of their children through contraceptive methods, infertility management, and informed reproductive-health decision-making (WHO, 2025). Modern contraceptives, such as injectable methods, oral contraceptives, implants, intrauterine devices, and barrier methods, are widely used because of their effectiveness. However, method choice is influenced by individual factors, access, counselling quality, and concerns about adverse effects. Hormonal contraceptives in particular have been associated with headaches, weight changes, mood alterations, irregular bleeding, fatigue, and delayed return of menses after discontinuation (Mukanga *et al.*, 2023), which may reduce acceptance and sustained use. Consequently, exploring alternative reproductive regulators from natural sources remains relevant for supporting the development of affordable, accessible options with fewer side effects.

Soybeans are a rich source of phytoestrogens, especially isoflavones, a class of polyphenolic plant compounds that are structurally similar to 17β-estradiol. This similarity enables isoflavones to bind estrogen receptors and to act as selective estrogen receptor

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modulators (Viscardi *et al.*, 2025). Their biological effects depend on exposure characteristics such as the type of phytoestrogen, concentration, bioavailability, and duration of exposure, as well as on hormonal status, physiological conditions, and tissue-specific responses (Domínguez-López *et al.*, 2020; Messina *et al.*, 2025). Soy isoflavones can interact with ERα and ERβ, producing estrogenic or antiestrogenic effects that vary by receptor subtype and tissue context (Raihanah *et al.*, 2024; Viscardi *et al.*, 2025). In the reproductive axis, hypothalamic–pituitary–ovarian regulation controls gonadotropin-releasing hormone (GnRH), luteinizing hormone (LH), follicle-stimulating hormone (FSH), estrogen production, ovulation, and follicle development (Reed & Carr, 2018). Through actions on estrogen receptors and related pathways, soy isoflavones have been investigated for effects on reproductive hormones and ovarian morphology (Raihanah *et al.*, 2024).

Previous animal studies have reported that soy isoflavones or soy-derived products can modulate reproductive hormones and ovarian function. For example, Ma *et al.* (2021) administered 100 mg/kg soy isoflavones for 28 days to a mouse model of polycystic ovary syndrome (PCOS) and observed improvements in oestrous cyclicity, ovarian morphology, and hormonal disturbances. Raihanah *et al.* (2024) similarly reported changes in FSH, estradiol, LH, testosterone, and the LH/FSH ratio in a PCOS model following isoflavone treatment. Experimental studies using doses of 50–100 mg/kg have also demonstrated effects on follicle development, oxidative stress, and reproductive-hormone regulation (Raihanah *et al.*, 2024). These findings suggest that soy isoflavones can influence ovarian function, particularly in hormonally perturbed states, although results vary with differences in animal models, dose, duration, reproductive status, and outcome measures.

Human studies yield mixed evidence on endocrine effects of soy isoflavones. Rizzo *et al.* (2022) reported that soy and isoflavone intake generally do not impair fertility in healthy women, while potential benefits were noted in some fertility-related conditions. A systematic review by Kang *et al.* (2022) found heterogeneous effects of isoflavone supplementation on menopausal symptoms and hormonal measures, including inconsistent results for FSH and LH. Likewise, Viscardi *et al.* (2025) reported no significant effects of soy isoflavones on several estrogenicity markers, including FSH and estradiol, in postmenopausal women. Together, these data indicate that isoflavone endocrine effects are modest and context-dependent, underscoring the need for further experimental clarification.

Most prior research has focused on PCOS models, menopausal populations, fertility-related outcomes, or general ovarian morphology; evidence remains limited regarding the effects of soybean-extract isoflavones on LH, FSH, and ovarian antral follicle diameter in healthy female mice (Mus musculus). Therefore, this study aimed to determine the effects of isoflavones derived from soybean extract on luteinizing hormone (LH) and follicle-stimulating hormone (FSH) levels, as well as ovarian antral follicle diameter, in female Mus musculus.

2. Materials and Methods

2.1. Study design and ethical approval

This laboratory-based experimental study used a posttest-only control group design. The independent variable was isoflavone administration; dependent variables were luteinizing hormone (LH) level, follicle-stimulating hormone (FSH) level, and ovarian antral follicle diameter. All animal procedures complied with ethical standards for biomedical research and were approved by the Medical/Health Research Bioethics Committee, Faculty of Medicine, Universitas Islam Sultan Agung, Semarang, Indonesia (Ethical Clearance No. 808/XII/2019/Komisi Bioetik, issued 20 December 2019).

2.2. Experimental animals

Fifteen healthy female *Mus musculus,* aged 90 days and weighing approximately 200 g, were used. Inclusion criteria were good health, active behaviour, normal physical activity, and absence of anatomical abnormalities. Animals that died during the study were excluded from analysis. Mice were acclimatised for 7 days prior to treatment, housed on bedding in ventilated cages, and maintained at ~27°C and ~77% relative humidity. Standard pellet feed (CP511) and water were provided ad libitum.

2.3. Sample size and group allocation

Sample size was calculated using Federer's

Table 1. Experimental Groups

Group	Description
K	Control group receiving oral aquadest
P1	Treatment group receiving 4.5 mg of isoflavone orally per administration
P2	Treatment group receiving 9 mg of isoflavone orally per administration

formula, yielding a minimum of five animals per group. Fifteen mice were randomised into three groups (n = 5 per group) (Table 1).

2.4. Isoflavone preparation and dosage

Isoflavones were obtained from fractionated soybean seed extract; soybean seeds were sourced from Semarang, Central Java, Indonesia. Solvents and reagents included distilled water, acetone, and absolute ethanol. Dosages were calculated using a human-to-rat conversion factor of 0.018 (Laurence and Bacharach). The P1 dose was derived from a 500 mg human-equivalent dose: (500 mg × 0.018) / 2 = 4.5 mg per administration. The P2 dose was derived from a 1000 mg human-equivalent dose: (1000 mg × 0.018) / 2 = 9 mg per administration. Doses were given orally twice daily for 15 days.

2.5. Treatment procedure and blood sample collection

Treatments were given for 15 consecutive days as described. On day 15, blood was collected in the morning via the orbital sinus. Approximately 1–2 mL of blood was allowed to clot for 1 hour, then centrifuged at 4000 rpm for 15 minutes. Serum was aliquoted and stored for LH and FSH assays.

2.6. Measurement of LH and FSH Levels

Serum LH and FSH were measured by enzyme-linked immunosorbent assay (ELISA). Absorbance was read at 450 nm using a spectrophotometer, and results were reported in pg/mL. Assays were performed at the Integrated Biomedical Laboratory, Faculty of Medicine, Sultan Agung Islamic University, Semarang, Indonesia

2.7. Histology and measurement of ovarian antral follicle diameter

After blood collection, ovaries were harvested, processed for histology, and examined under an Olympus light microscope at 100× total magnification (10× objective, 10× ocular). Antral follicle diameters were measured in micrometres (μm). Representative histological images were used to illustrate antral follicle morphology for each group.

2.8. Data Analysis

Data were analysed using SPSS version 24. Descriptive statistics summarised LH, FSH, and antral follicle diameter per group. Normality was assessed with the Shapiro–Wilk test (sample size <50); homogeneity of variance was assessed by Levene's test. Variables that failed the normality or homogeneity assumptions were compared between groups using the Kruskal–Wallis test. A two-sided p-value <0.05 was considered statistically significant.

3. Results

Descriptive statistics for LH, FSH, and ovarian

Table 2. Descriptive statistics for LH and FSH serum levels and ovarian antral follicle diameter by treatment group

Variable	Group	Mean ± SD	Median	Min-Max	Kruskal-Wallis *p*-value
LH Level (pg/mL)	K	57.60 ± 3.65	58.00	53.00 – 62.00	0.371
	P1	59.80 ± 2.17	61.00	56.00 – 61.00	
	P2	60.40 ± 3.58	61.00	55.00 – 64.00	
FSH Level (pg/mL)	K	33.00 ± 7.52	31.00	26.00 – 44.00	0.570
	P1	35.60 ± 11.33	37.00	24.00 – 48.00	
	P2	28.00 ± 3.00	29.00	23.00 – 31.00	
Ovarian antral follicle diameter (μm)	K	278.35 ± 37.80	265.12	229.81 – 324.63	0.543
	P1	342.72 ± 167.64	241.04	204.13 – 528.05	
	P2	347.07 ± 62.72	383.80	261.42 – 403.48	

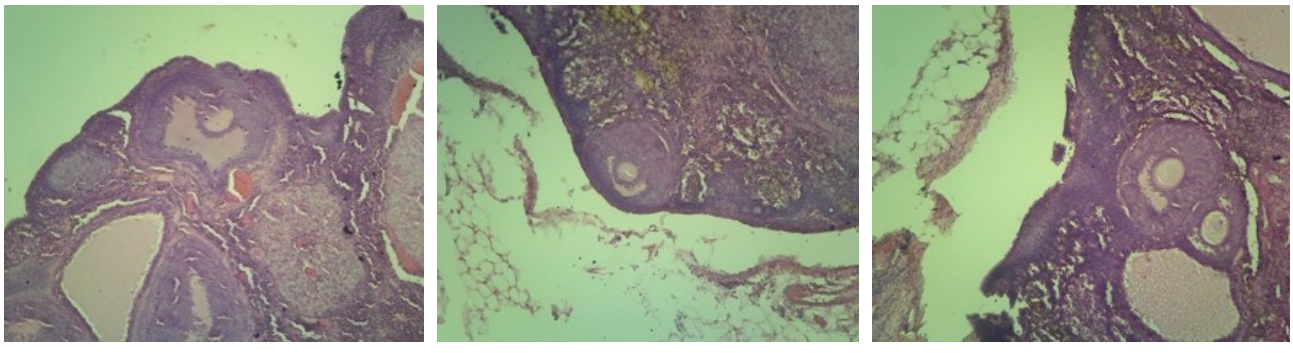

Figure 1. Histological images of ovarian antral follicle diameter

antral follicle diameter are summarised in Table 2. Group K (control) received oral aquadest; P1 received 4.5 mg isoflavone per administration; P2 received 9 mg isoflavone per administration. The mean LH concentrations were 57.60 ± 3.65 pg/mL (K), 59.80 ± 2.17 pg/mL (P1), and 60.40 ± 3.58 pg/mL (P2). Median LH values were 58.00 pg/mL (K), 61.00 pg/mL (P1), and 61.00 pg/mL (P2). Although mean LH was slightly higher in the isoflavone-treated groups, the difference was not statistically significant (Kruskal–Wallis H = 1.982, p = 0.371). Mean FSH levels were 33.00 ± 7.52 pg/mL (K), 35.60 ± 11.33 pg/mL (P1), and 28.00 ± 3.00 pg/mL (P2). Median FSH values were 31.00 pg/mL (K), 37.00 pg/mL (P1), and 29.00 pg/mL (P2). Between-group differences in FSH were not significant (Kruskal–Wallis H = 1.123, p = 0.570). Mean ovarian antral follicle diameters were 278.35 ± 37.80 µm (K), 342.72 ± 167.64 µm (P1), and 347.07 ± 62.72 µm (P2). Median diameters were 265.12 µm (K), 241.04 µm (P1), and 383.80 µm (P2). No significant differences were observed among groups (Kruskal–Wallis H = 1.220, p = 0.543). Normality testing (Shapiro–Wilk) indicated a non-normal distribution for LH in P1 and antral follicle diameter in P1; Levene's test showed unequal variances for FSH and antral follicle diameter. Consequently, nonparametric between-group comparisons (Kruskal–Wallis) were applied. Overall, isoflavone administration at the tested doses did not produce statistically significant changes in LH, FSH, or ovarian antral follicle diameter in female mice. Representative histological images of ovarian antral follicles for each group are presented in Figure 1.

3.1. Luteinizing hormone (LH) levels

Figure 2 shows the distribution of LH levels for control (K), P1, and P2. Median LH was 58.00 pg/mL (range 53.00–62.00) in K, 61.00 pg/mL (mostly 60.00–61.00; one outlier at 56.00) in P1, and 61.00 pg/mL (range 55.00–64.00) in P2. Although medians were higher in the isoflavone-treated groups, distributions overlapped across groups. The Kruskal–Wallis test indicated no significant difference among groups (H = 1.982, df = 2, p = 0.371), suggesting that the tested isoflavone doses did not significantly affect LH levels in female mice.

3.2. Follicle-Stimulating Hormone (FSH) Levels

The distribution of FSH levels among the control group (K), treatment group 1 (P1), and treatment group 2 (P2) is shown in Figure 3. The median FSH level in the control group was 31.00 pg/mL, with values ranging from 26.00 to 44.00 pg/mL. In P1, the median FSH level was higher, at 37.00 pg/mL, with a wider data distribution ranging from 24.00 to 48.00 pg/mL. This indicates greater variability of FSH levels in the P1 group compared with the other groups. In P2, the median FSH level was 29.00 pg/mL, with most values clustered around 28.00–29.00 pg/mL. However, two outlying values were observed in this group, namely one lower value at 23.00 pg/mL and one higher value at 31.00 pg/mL. Descriptively, FSH levels appeared to increase in P1 compared with the control group, but decreased in P2.

Despite these descriptive differences, the Kruskal–Wallis test showed no statistically significant difference

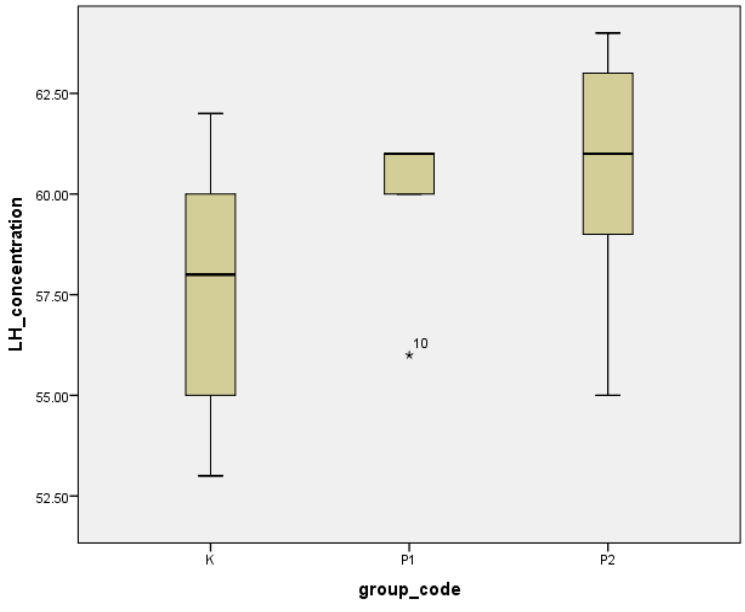

Figure 2. Boxplot of serum LH levels (pg/mL) by group

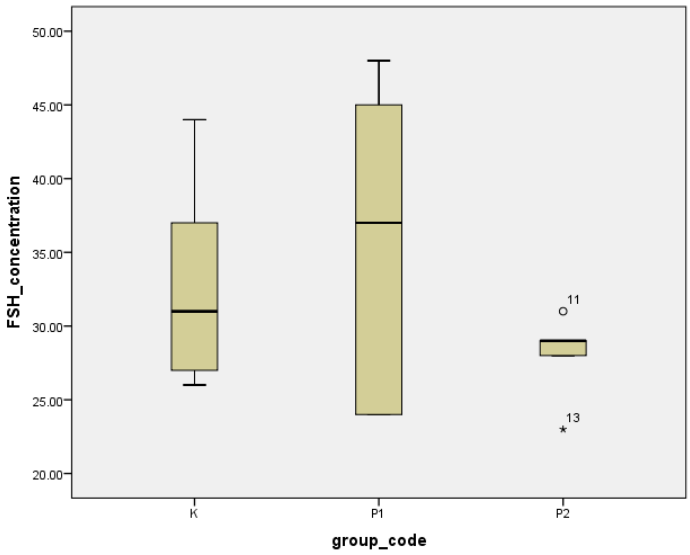

Figure 3. Boxplot of FSH levels among groups

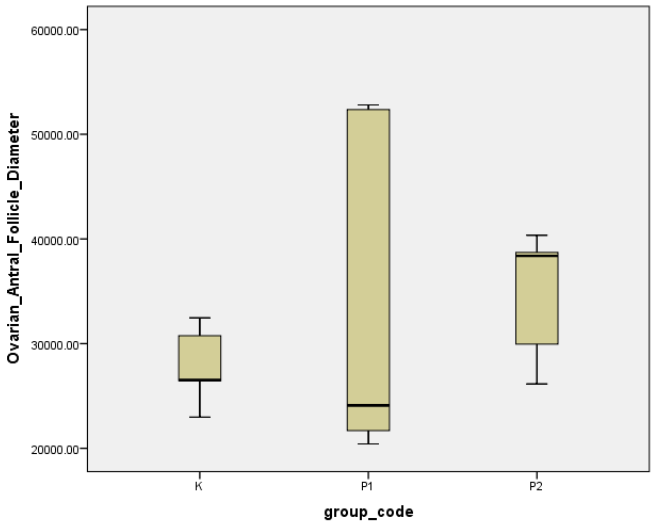

Figure 4. Boxplot ovarian antral follicle diameter

in FSH levels among the three groups (H = 1.123, df = 2, p = 0.570). These findings indicate that isoflavone administration at the given doses did not significantly alter FSH levels in female mice.

3.3. Ovarian Antral Follicle Diameter

The distribution of ovarian antral follicle diameters is shown in Figure 4. Median (range) diameters were: control (K) 265.12 µm (229.81–324.63), P1 241.04 µm (204.13–528.05), and P2 383.80 µm (261.42–403.48). Although P2 exhibited the highest median diameter and P1 the greatest variability, values overlapped across groups. The Kruskal–Wallis test indicated no significant difference among groups (H = 1.220, df = 2, p = 0.543), suggesting that isoflavone treatment at these doses did not significantly alter ovarian antral follicle diameter.

4. Discussion

This study evaluated the effects of soybean extract isoflavones on LH, FSH, and ovarian antral follicle diameter in healthy female mice. The main finding was that 15 days of oral isoflavone administration at the tested doses did not produce statistically significant differences in LH (p = 0.371), FSH (p = 0.570), or antral follicle diameter (p = 0.543) among control, P1, and P2 groups. Although some descriptive differences were observed, slightly higher median LH in treated groups, the highest median FSH and greatest variability in P1, and the largest median follicle diameter in P2. These differences overlapped across groups and were not statistically robust.

The lack of significant change in LH and FSH suggests that, under the conditions tested, isoflavone exposure did not meaningfully alter gonadotropin

secretion. Isoflavones are phytoestrogens that can interact with estrogen receptors and potentially influence reproductive endocrine regulation, but their effects depend on dose, exposure duration, animal model, hormonal milieu, and tissue-specific receptor responses. Prior studies indicate that the effects of isoflavones are more evident in models with pre-existing endocrine disturbance; for instance, soy isoflavones have improved hormone balance and ovarian morphology in PCOS models (Ma *et al.*, 2021; Raihanah *et al.*, 2024). A systematic review and meta-analysis also reported no significant effect of isoflavone supplementation on LH or FSH overall (Kang *et al.*, 2022), consistent with our results.

The absence of a significant difference in antral follicle diameter, despite a higher median in P2 and wide variability in P1, likely reflects biological variability and study constraints. Follicular size is influenced by FSH dynamics, oestrous cycle stage, and individual variability; thus, single end-point measurements in unsynchronised healthy animals may obscure subtle treatment effects. Histology confirmed antral follicles across groups, but quantitative differences were not detected.

Several study limitations likely contributed to the null findings. Treatment duration (15 days) may have been insufficient to elicit measurable endocrine or histological changes in healthy mice. The small sample size (n = 5 per group) limited statistical power and increased sensitivity to individual variation. The oestrous cycle phase was not synchronised at sampling, allowing cycle-related fluctuations in gonadotropin and follicle measurements. Finally, the absence of measurements for estradiol, progesterone, GnRH, isoflavone metabolites, and estrogen receptor expression restricts mechanistic interpretation.

Strengths of the study include a controlled experimental design with two dose groups, combined biochemical (ELISA) and histological assessments, and transparent reporting of variability. For future research, we recommend larger sample sizes, longer exposure periods, synchronisation of the oestrous cycle at sampling, and inclusion of additional endocrine and molecular endpoints (estradiol, progesterone, GnRH, isoflavone metabolite levels, estrogen receptor expression). Such measures would improve power to detect treatment effects and clarify mechanisms by which isoflavones may influence the hypothalamic–pituitary–ovarian axis, particularly in pathological models (e.g., PCOS) where prior studies suggest benefit (Ma *et al.*, 2021; Raihanah *et al.*, 2024).

5. Conclusions

Oral administration of soybean extract isoflavones (4.5 mg and 9 mg per administration) for 15 consecutive days did not produce statistically significant changes in LH, FSH, or ovarian antral follicle diameter in female mice (*Mus musculus*). Although descriptive differences were observed among groups, these were not supported by the Kruskal–Wallis's test. Future studies with larger sample sizes, longer treatment periods, oestrous cycle synchronisation, and additional endocrine and molecular endpoints are recommended to more definitively assess the effects of isoflavones on the hypothalamic–pituitary–ovarian axis and follicular development.

Conflict of interest

The authors declare no conflicts of interest.

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