RESEARCH ARTICLE

Human Umbilical Cord-Derived Mesenchymal Stem Cell Secretome Modulates Ovarian TNF-α, Oxidative Stress, and Serum Estradiol in Letrozole-Induced PCOS Rats

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Abstract

BACKGROUND: Polycystic ovary syndrome (PCOS) is characterized by hyperandrogenism, anovulation, chronic inflammation, and oxidative stress. Current treatments mainly relieve symptoms without addressing ovarian damage. Human umbilical cord–derived mesenchymal stem cell (hUC-MSC) secretome is a cell-free product with anti-inflammatory, antioxidant, and regenerative effects that avoids cell-related risks, but its role in PCOS remains unclear. This study was conducted to evaluate its effects on ovarian tumor necrosis factor (TNF)-α, oxidative stress, and serum estradiol in letrozole-induced PCOS rats.

METHODS: Thirty female Wistar rats were assigned to a normal group, untreated PCOS-induced group, and PCOS-induced groups treated with either 200, 300, or 400 µL hUC-MSC secretome. PCOS was induced with letrozole for 21 days, and confirmed by vaginal cytology on day-22. Ovarian TNF-α expression, serum estradiol, ovarian malondialdehyde (MDA), and ovarian superoxide dismutase (SOD) activity were measured using reverse transcription quantitative polymerase chain reaction (RT-qPCR), enzyme-linked immunosorbent assay (ELISA), the thiobarbituric acid reactive substances (TBARS) assay, and the water-soluble tetrazolium salt (WST) assay, respectively.

RESULTS: Letrozole successfully induced PCOS characteristics, which was marked by persistent diestrus and ovarian histological alterations in PCOS-induced groups. Compared with the untreated PCOS group, group receiving 200 µL hUC-MSC secretome showed lower ovarian TNF-α expression and MDA levels, as well as higher SOD activity and serum estradiol concentrations, approaching normal control values, although these differences were not statistically significant. Compared with the other hUC-MSC concentrations, the 200 µL dose showed the most consistent trend across inflammatory, oxidative stress, and hormonal parameters.

CONCLUSION: Administration of hUC-MSC secretome, especially 200 µL dose, showed a trend toward reduced ovarian inflammation and oxidative stress in PCOS rats. These findings suggest that hUC-MSC secretome may have potential as a complementary treatment for PCOS.

KEYWORDS: polycystic ovary syndrome, hUC-MSC secretome, TNF-α, oxidative stress, estradiol

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Introduction

Polycystic ovary syndrome (PCOS) is a multifactorial disorder associated with impaired reproductive and metabolic function.(1) Beyond its hallmark features of hyperandrogenism and ovulatory dysfunction, the syndrome is often accompanied by insulin resistance, obesity, dyslipidemia, and other cardiometabolic abnormalities. Emerging evidence suggests that interactions among endocrine dysregulation, chronic inflammation, and oxidative stress contribute substantially to disease development and progression.(2)

Among the inflammatory mediators involved in PCOS, tumor necrosis factor (TNF)-α plays a central role in the chronic low-grade inflammation characteristic of the disorder.(3) Excessive TNF-α signaling contributes to ovarian dysfunction through impairment of granulosa cell survival and steroidogenic activity, ultimately interfering with normal follicular development and ovulation.(3) Furthermore, TNF-α participates in a broader inflammatory network by promoting downstream cytokine product ion, thereby sustaining chronic ovarian inflammation.(3,4) This inflammatory state is closely linked to oxidative stress, as TNF-α stimulates the generation of reactive oxygen species (ROS) in ovarian cells.(4)

Oxidative imbalance in PCOS arises when ROS generation exceeds the capacity of endogenous antioxidant defenses. Persistent oxidative stress damages cellular macromolecules and compromises ovarian function, creating conditions that further aggravate inflammatory signaling.(4,5) Elevated malondialdehyde (MDA) and altered superoxide dismutase (SOD) activity are among the most frequently reported biomarkers of oxidative imbalance in PCOS.(5) Persistent oxidative stress also amplifies inflammatory signaling, establishing a pathological inflammatory-oxidative cycle that contributes to disease progression.(4)

Inflammation and oxidative stress directly affect ovarian steroidogenesis. Excessive ROS suppresses aromatase activity and impairs the conversion of androgens into estradiol, resulting in reduced estrogen production and impaired follicular development.(6) Consequently, simultaneous evaluation of TNF-α, oxidative stress biomarkers, and estradiol may provide a comprehensive assessment of ovarian function in PCOS. Since these three components are mechanistically linked, a therapeutic effect on one pathway may influence the others. However, previous studies of MSC-derived secretome in PCOS models have generally assessed them separately. Simultaneous evaluation of TNF-α, oxidative stress biomarkers, and estradiol in the same animals providing a more comprehensive assessment of ovarian function in PCOS than any single parameter alone.

Current treatments for PCOS mainly relieve symptoms without addressing ovarian damage. Human umbilical cord-derived mesenchymal stem cell (hUC-MSC) secretome exerts its biological actions through a complex repertoire of bioactive molecules, including soluble proteins, immunomodulatory mediators, extracellular vesicles, and regulatory nucleic acids, which collectively contribute to tissue repair mechanisms.(7,8) A previous study reported that MSC-derived secretome reduced inflammatory cytokine expression and improved ovarian morphology in an experimental model of PCOS.(9) Despite these findings, evidence regarding the integrated effects of hUC-MSC secretome on ovarian inflammation, oxidative stress, and endocrine function in letrozole-induced PCOS is still limited. Therefore, this post-test controlled quasi-experimental study was performed to assess the effects of hUC-MSC secretome on these parameters in PCOS rats model.

Methods

Animals and Housing

Thirty female Wistar rats (*Rattus norvegicus*; 6-8 weeks old; 120-150 g) were obtained from the Kemuning Laboratory Animal Center, Karanganyar, Indonesia. Animals were housed under controlled conditions (22±2°C, 40-60% humidity, 12:12 h light-dark cycle) with free access to standard phytoestrogen-free chow and water.(3) All procedures complied with institutional animal care guidelines and the American Veterinary Medical Association (AVMA) Guidelines for the Euthanasia of Animals (2020), and ethical approval was obtained before study initiation.(4,5) All procedures related to animal husbandry, intervention, and euthanasia were also performed in compliance with institutional ethical standards and the principles of Replacement, Reduction, and Refinement (3Rs). All experimental procedures were conducted at the Integrated Research and Testing Laboratory (LPPT), Universitas Gadjah Mada, Indonesia.

Experimental Design and Group Allocation

Following acclimatization, the animals were randomly allocated into five groups: normal control rats (Normal),

untreated PCOS-induced rats (PCOS), and PCOS-induced rats treated with hUC-MSC secretome at 200 µL (Sec200), 300 µL (Sec300), or 400 µL (Sec400). Sample size was calculated using the resource equation method (n = DF/k + 1, with DF = 20 and k = 5), yielding five rats per group; one rat was added to each group to anticipate a 20% dropout (n = 6 per group). No positive control was included, as this study aimed to evaluate the dose-dependent effects of the secretome relative to untreated PCOS rats. The secretome was administered intraperitoneally on day-23, -25, -28, and -30 after letrozole induction.(10) Doses were based on a previous hUC-MSC secretome study in PCOS rats (10), with three escalating doses to assess a dose–response relationship, and all volumes were within recommended intraperitoneal limits for rats (11). The injection interval was chosen to allow ovarian tissue regeneration and immune response to the secretome's bioactive factors.(12)

PCOS Induction with Letrozole

PCOS was induced by oral administration of 1 mg/kg body weight/day letrozole (Letrozole®, Lebrest; PT Ferron Par Pharmaceuticals, Bekasi, Indonesia) suspended in 0.5% carboxymethylcellulose (CMC) once daily for 21 consecutive days (day-1 to day-21). Dosages were adjusted according to individual body weight throughout the induction period, while control animals received an equivalent volume of 0.5% CMC vehicle.

Estrous Cytology Assessment

Estrous cycle stages were determined by vaginal cytology using a modified protocol based on previous publication. (13) Vaginal lavage was performed with approximately 0.2 mL sterile normal saline, and collected cells were examined under a light microscope (400×) without staining. Estrous stages were identified according to the predominant cell type, including nucleated epithelial cells (proestrus), cornified epithelial cells (estrus), and leukocyte-dominant smears (metestrus/diestrus).(14) Daily smears were obtained from day-14 to day-21. Persistent diestrus, defined as ≥7 consecutive days of leukocyte-dominant smears, was used as an indicator of anovulatory arrest and successful PCOS induction.(15) Rats exhibiting persistent diestrus or irregular estrous cycles after 21 days were considered successfully induced, whereas control rats maintained a normal 4-5 day estrous cycle.(15,16)

hUC-MSC Secretome Preparation and Administration

Regenic® (PT Bifarma Adiluhung, Jakarta, Indonesia), the secretome used in this study, was derived from cultured hUC-MSCs and contains a variety of bioactive factors that contribute to paracrine-mediated regenerative effects.(7-9) The hUC-MSC secretome was administered intraperitoneally at doses of 200, 300, or 400 µL on day-23, -25, -28, and -30 following PCOS induction. The normal control and untreated PCOS groups received intraperitoneal injections of 200 µL of distilled water at the same time points as the secretome-treated groups (day-23, -25, -28, and -30) to control for injection-related stress. Animals were euthanized on day-33 for subsequent analyses.

Sacrifice and Tissue Collection

One additional rat per group (5 in each group, 35 rats overall) was euthanized on day-22 to confirm PCOS induction histologically; these animals were excluded from analysis, leaving six rats per group (30 in total) for treatment and final evaluation. The remaining animals (n = 30) were euthanized on day-33 after treatment completion. Euthanasia was performed under intraperitoneal 80-100 mg/kg ketamine anesthesia.(17,18) Blood and ovarian tissues were collected for hormonal, molecular, and biochemical analyses.(11) Carcasses were disposed of according to institutional biosafety regulations.

Histological Evaluation of Ovarian Tissue

Ovaries collected on day-22 were examined histologically to confirm PCOS induction based on characteristic features, including cystic follicles, granulosa cell thinning, reduced corpus luteum formation, and stromal thickening.(16) Histological findings were compared between control and letrozole induced animals prior to treatment. Day-33 histological evaluations were performed but not included as primary outcomes.

RNA Extraction and Quantitative Real-Time PCR Analysis

Gene expression profiling was conducted by evaluating the ovarian tissue-derived RNA. Following RNA isolation and cDNA synthesis in accordance with the manufacturer's protocol (Cat. No. RK20433-100RXN; ABclonal Technology, Wuhan, China), the abundance of the TNF-α transcript was measured by reverse transcription quantitative polymerase chain reaction (RT-qPCR). Relative gene expression was calculated using the comparative $2^{-\Delta\Delta Ct}$ approach, with β-actin employed as the endogenous control for normalization. (19) The primer sequence for TNF-α was as follows: Forward: AACACACGAGACGCTGAAGT; Reverse: TCCAGTGAGTTCCGAAAGCC; meanwhile, for β-actin

was as follows: Forward: CATGTACGTAGCCATCCA-GGC; Reverse: CTCTTTAATGTCACGCACGAT.

Measurement of SOD Activity

Assessment of ovarian antioxidant status was performed through determination of SOD activity using a WST-based colorimetric assay (Cat No. E-BC-K020-M; Elabscience Biotechnology, Wuhan, China). Enzymatic activity was measured spectrophotometrically at 450 nm and subsequently adjusted for total protein content. Final results were reported as U/mg protein.(20)

Measurement of MDA Concentration

Lipid peroxidation within ovarian tissue was evaluated through measurement of MDA concentration using a TBARS-based assay (Cat. No. E-BC-K025-S; Elabscience Biotechnology). Following reaction with the assay reagents and heat-induced derivatization, samples were analyzed spectrophotometrically at 532 nm. MDA values were normalized to protein content and reported as nmol/mg protein.(21)

Measurement of Serum Estradiol

Serum estradiol was quantified by enzyme-linked immunosorbent assay (ELISA) using a commercially available kit (Cat. No. E-EL-0152; Elabscience Biotechnology). Concentrations were derived from a standard calibration curve generated within each analytical run and were presented as pg/mL.(22)

Statistical Analysis

Statistical analysis was performed using IBM SPSS Statistics (IBM Corporation, Armonk, NY, USA). Data normality was assessed using the Shapiro-Wilk test, and homogeneity of variance using Levene's test. Ovarian TNF-α expression and SOD activity met parametric assumptions and were analyzed using one-way ANOVA followed by Tukey's HSD post hoc test. Ovarian MDA levels and serum estradiol concentrations did not meet the normality assumption and were analyzed using the Kruskal-Wallis test. Data are presented as mean±SD, and statistical significance was set at $p<0.05$.

Results

Validation of the PCOS Model

Successful PCOS induction was confirmed by evaluation of vaginal cytology and ovarian histopathology. Vaginal cytology that was monitored from day-14 to -21 showed that all rats letrozole-administered rats (24 from 24, 100%) exhibited persistent diestrus with leukocyte-dominant vaginal smears for at least 7 consecutive days, whereas the normal control rats (0 from 6, 0%) maintained regular estrous cycles. PCOS induction was confirmed on day-22 before hUC-MSC secretome administration. The representative cytological findings corresponding to each phase of the estrous cycle were shown in Figure 1, with black arrows indicating the dominant cell population.

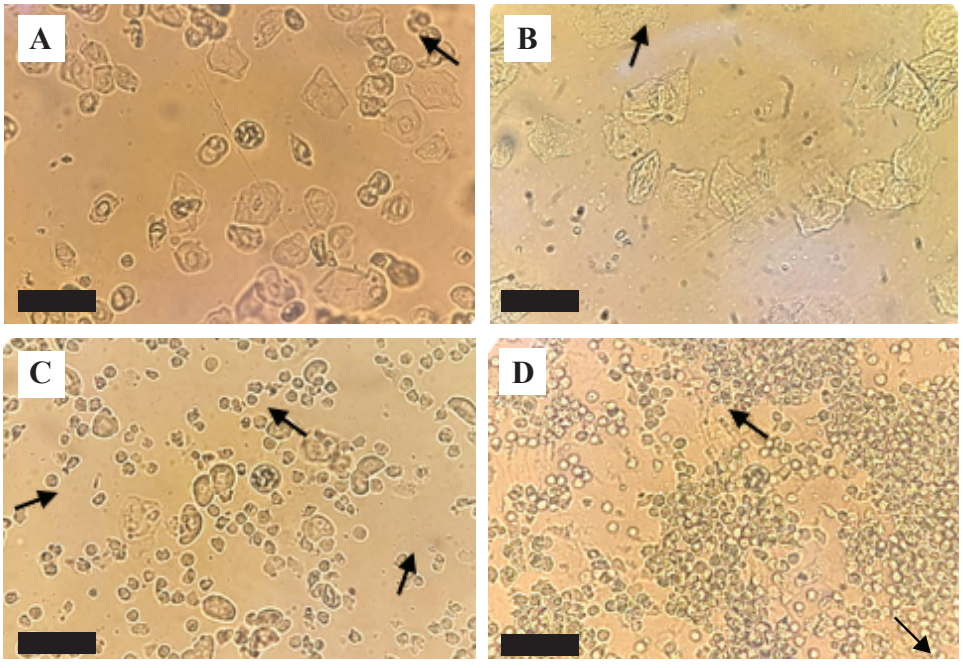


Figure 1. Representative vaginal cytology corresponding to the different stages of the rat estrous cycle. A: Proestrus characterized by abundant nucleated epithelial cells. B: Estrus, dominated by cornified epithelial cells. C: Metestrus, showing a mixed cellular composition consisting of nucleated epithelial cells, cornified epithelial cells, and leukocytes. D: Diestrus, characterized by leukocyte predominance. Black arrows indicate the dominant cell population observed in each stage. Vaginal samples were collected by lavage using sterile 0.9% saline and examined microscopically for estrous cycle determination and identification of persistent diestrus following the letrozole administration. Black bar: 50 µm.

Histological findings further confirmed successful induction of the PCOS phenotype. Representative ovarian sections were shown in Figure 2. Ovaries from the normal control group displayed follicles at various developmental stages and multiple corpus luteum structures, indicating normal ovulatory activity. In contrast, ovaries from letrozole-induced animals demonstrated characteristic PCOS morphology, including multiple cystic follicles, thinning of the granulosa cell layer, stromal thickening, and reduced corpus luteum formation. These findings collectively confirmed successful induction of the experimental PCOS model prior to hUC-MSC secretome administration.

Administration of hUC-MSC Secretome Reduced Ovarian TNF-α Expression

Relative ovarian TNF-α expression was measured as an indicator of ovarian inflammatory activity following hUC-MSC secretome administration. The distribution of TNF-α expression across experimental groups was shown in Figure 3. Elevated TNF-α expression was observed in the untreated PCOS group relative to the other experimental groups, supporting the presence of ovarian inflammatory activation following letrozole induction. TNF-α expression was numerically lower in secretome-treated groups, especially Sec200, but differences among groups were not statistically significant. This may reflect the complex composition of the secretome, in which different bioactive molecules can exert varying effects on inflammatory pathways. Furthermore, the anti-inflammatory response may not be directly dose-dependent, as higher concentrations do not necessarily produce greater therapeutic effects. However, because the differences were not statistically significant, these explanations remain hypothetical and require further investigation (p=0.17).

Administration of hUC-MSC Secretome Differentially Affected Ovarian SOD Activity

Ovarian SOD activity was evaluated to assess the antioxidant response following hUC-MSC secretome treatment. The activity profile of SOD across experimental groups was illustrated in Figure 4. Differences in ovarian SOD activity across experimental groups were modest. The untreated PCOS group displayed numerically greater SOD activity than normal controls, whereas secretome-treated animals exhibited a variable response pattern across doses. Activity levels were highest in Sec200, lower in Sec300, and increased again in Sec300, yielding values similar to those observed in normal animals. Statistical comparison showed that these variations did not reach significance (p=0.55).

Administration of hUC-MSC Secretome Differentially Affected Ovarian MDA Concentration

Ovarian malondialdehyde (MDA) concentration was measured to evaluate lipid peroxidation and oxidative stress status following hUC-MSC secretome administration. The distribution of MDA concentrations among experimental groups was illustrated in Figure 5. The PCOS group showed a slightly higher median ovarian MDA concentration than the Normal group. Among the secretome-treated groups, Sec200 showed the lowest median, Sec300 the highest

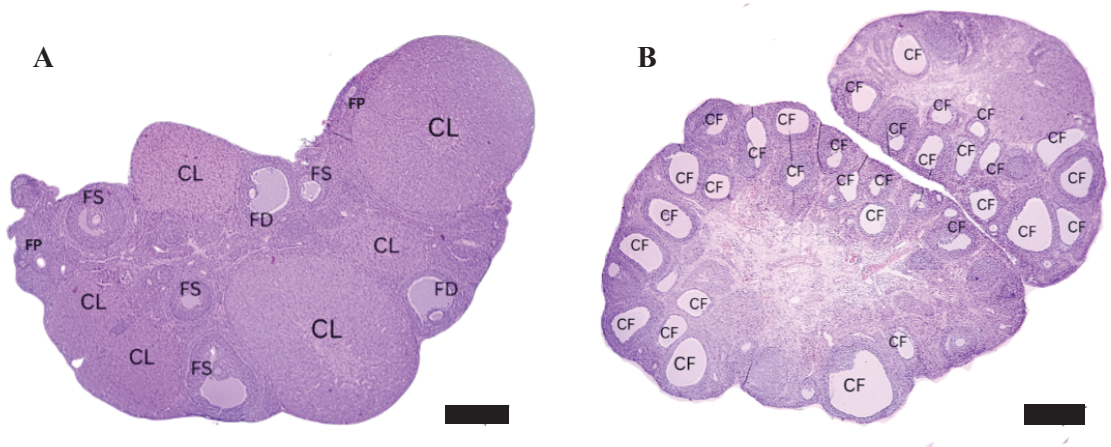


Figure 2. Ovarian histology of the normal control (A) and letrozole-induced PCOS (B) groups stained with hematoxylin and eosin (H&E). Ovaries from the normal control group displayed preserved follicular architecture, including primary follicles (PF), secondary follicles (SF), Graafian follicles (GF), and corpus luteum (CL), indicating ongoing ovulatory activity. Ovarian sections from the PCOS group showed multiple cystic follicles (CF) accompanied by enlarged follicular spaces, attenuation of the granulosa cell layer, and reduced corpus luteum structures, consistent with disrupted follicular maturation and anovulation. Black bar: 200 µm.

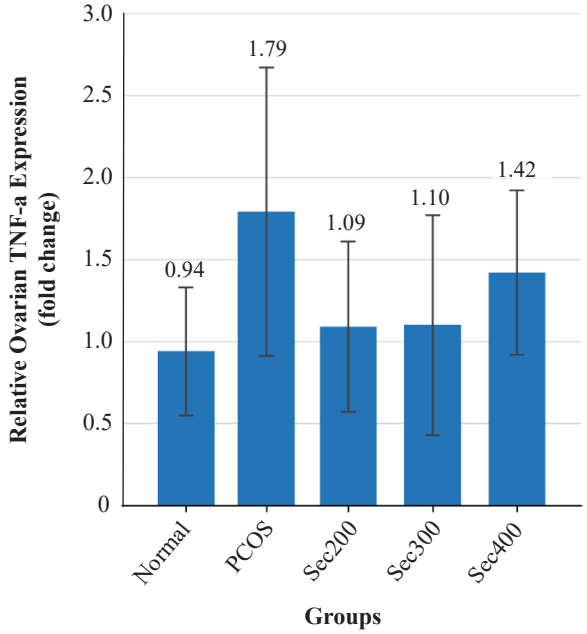


Figure 3. Relative ovarian TNF-α expression in the experimental groups. Relative gene expression was quantified by RT-qPCR using the $2^{-\Delta\Delta Ct}$ method with β-actin as the reference gene and is presented as mean±SD. Normal: normal control; PCOS: negative control; Sec200: PCOS + 200 µL secretome; Sec300: PCOS + 300 µL secretome; Sec400: PCOS + 400 µL secretome. Statistical analysis was performed using One-way ANOVA, showing no significant difference among groups (p=0.17).

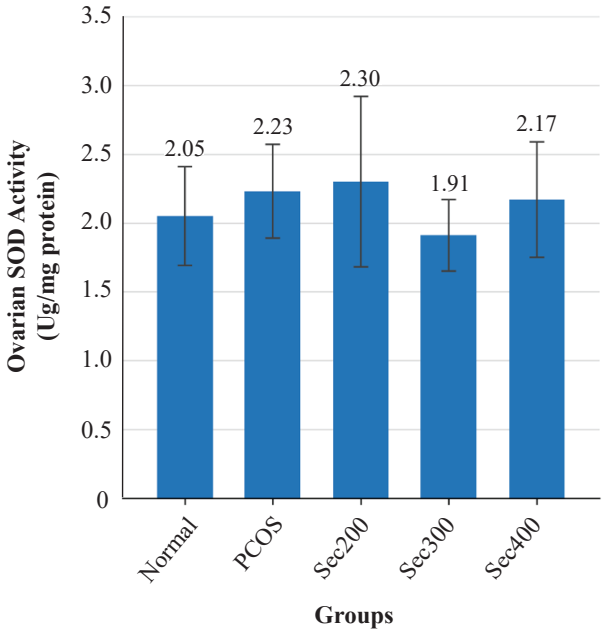


Figure 4. Ovarian superoxide dismutase (SOD) activity in the experimental groups. SOD activity was measured in ovarian tissue homogenates using a WST-based colorimetric assay. Data are presented as individual values with mean±SD. Normal: normal control; PCOS: negative control; Sec200: PCOS + 200 µL secretome; Sec300: PCOS + 300 µL secretome; Sec400: PCOS + 400 µL secretome. Statistical analysis using One-way ANOVA, showing no significant difference among groups (p=0.55).

median with the widest data range, and Sec400 a median between those of Sec200 and Sec300. Individual values overlapped considerably across groups, and no significant differences were found (Kruskal–Wallis, p=0.31) (Figure 5). Interquartile ranges and individual values overlapped considerably among groups, suggesting substantial variability and contributing to the absence of statistically significant differences (Kruskal-Wallis, p=0.31).

Among the secretome-treated groups, Sec200 showed the most favorable numerical trends for oxidative stress parameters, which show lower MDA levels and higher SOD activity. However, the response varied across doses, as SOD activity decreased in Sec300, suggesting a potentially non-linear dose response. These findings indicate that hUC-MSC secretome, particularly at 200 µL, may have potential to modulate ovarian inflammation and oxidative stress in PCOS, although this effect might not be linear with the dosage.

Administration of hUC-MSC Secretome Differentially Affected Serum Estradiol Concentration

Serum estradiol concentrations were measured to evaluate ovarian endocrine function following hUC-MSC secretome administration. The distribution of serum estradiol concentrations among experimental groups was presented in Figure 6. The PCOS group showed a lower median estradiol concentration than the normal control group. Secretome treated groups generally exhibited higher median estradiol concentrations than the PCOS group, particularly Sec200, which showed the highest median value among treatment groups. Median estradiol concentration in the Sec400 group also appeared higher than that of the PCOS group, whereas Sec300 showed a relatively lower median value. The wide within-group variability may partly reflect differences in estrous cycle stage at sampling, as estrous cycles were not synchronized before induction. Interquartile ranges and individual values overlapped considerably among groups, indicating substantial within group variability and contributing to the absence of statistically significant differences (Kruskal-Wallis, p=0.59).

Discussion

Persistent diestrus was observed in all letrozole treated animals, whereas normal cyclicity was maintained in the

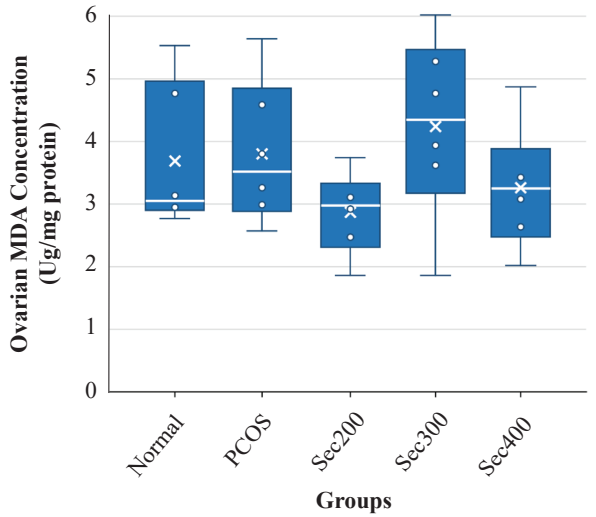


Figure 5. Ovarian MDA concentration in the experimental groups. MDA concentration was measured in ovarian tissue homogenates. Boxplots represent the median and interquartile range (IQR), with individual data points shown. Normal: normal control; PCOS: negative control; Sec200: PCOS + 200 µL secretome; Sec300: PCOS + 300 µL secretome; Sec400: PCOS + 400 µL secretome. Statistical analysis using the Kruskal-Wallis test, showing no significant difference among groups (p=0.31).

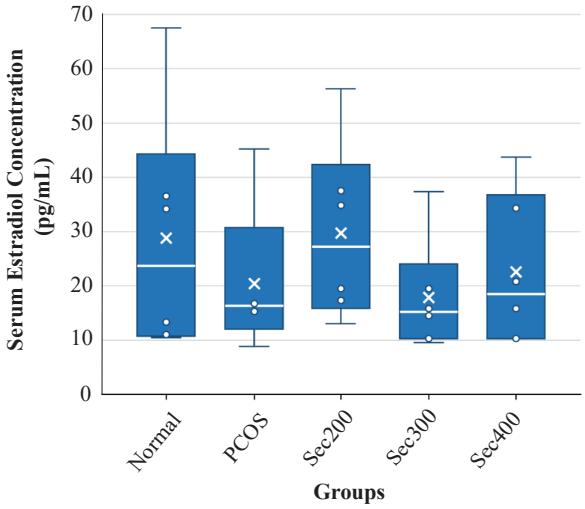


Figure 6. Serum estradiol concentrations in the experimental groups. Serum estradiol levels were determined by ELISA. Boxplots represent the median and interquartile range (IQR), with individual data points shown. Normal: normal control; PCOS: negative control; Sec200: PCOS + 200 µL secretome; Sec300: PCOS + 300 µL secretome; Sec400: PCOS + 400 µL secretome. Statistical analysis using the Kruskal-Wallis test, showing no significant difference among groups (p=0.59).

control group. Histological examination revealed multiple cystic follicles, granulosa cell thinning, stromal thickening, and reduced corpus luteum formation, indicating successful reproduction of key reproductive features of PCOS. Letrozole inhibits aromatase activity, resulting in androgen accumulation, impaired follicular maturation, and anovulation.(23) Alterations in hypothalamic-pituitary-ovarian axis signaling further contribute to reproductive dysfunction, supporting previous findings that chronic letrozole exposure reproduces PCOS-like endocrine abnormalities.(24) Histological alterations observed in this study are characteristic of letrozole induced PCOS. Enlarged cystic follicles and reduced corpus luteum structures indicate follicular arrest and chronic anovulation, whereas granulosa cell thinning and stromal thickening are associated with hormonal imbalance, ovarian remodeling, and increased androgen production.(23,25)

Ovarian TNF-α expression was evaluated as a marker of ovarian inflammation. The negative control group exhibited the highest TNF-α expression, whereas lower values were observed following hUC-MSC secretome administration, though not statistically significant. The observed pattern is consistent with reduced ovarian inflammatory activity after treatment. Elevated TNF-α expression is a characteristic feature of PCOS and has been associated with impaired granulosa cell function, disrupted follicular maturation, and altered steroidogenesis.(25,26) Activation of Nuclear factor-kappa B (NF-Kb) signaling further promotes a pro-inflammatory ovarian environment.(27) Ovarian TNF-α expression is therefore widely used as an indicator of local inflammatory status in PCOS.

Lower TNF-α expression in secretome-treated animals may reflect the immunomodulatory effects of hUC-MSC derived bioactive factors, including soluble mediators, extracellular vesicles, growth factors, and cytokines.(7,28) Previous studies have shown that MSC-derived secretome suppresses pro-inflammatory cytokine production, limits NF-κB activation, and promotes resolution of inflammation.(29,30) The lowest TNF-α expression was observed in Sec200, suggesting that the 200 µL dose produced the most consistent biological response. Interpretation of this finding should remain cautious because substantial inter-animal variability was present, a phenomenon commonly reported in experimental PCOS models.(25) The absence of statistical significance may also be influenced by the lack of biodistribution analysis and absence of estrous cycle synchronization before treatment and tissue collection. The overall expression pattern suggests a possible anti-inflammatory trend of hUC-MSC secretome in PCOS, although the differences were not statistically significant.

Accumulating evidence indicates that oxidative stress contributes to ovarian dysfunction in PCOS through

disruption of follicular maturation, steroidogenesis, and inflammatory regulation.(4,5) Ovarian SOD activity and MDA concentration were therefore evaluated to assess redox homeostasis following secretome treatment. No significant differences were observed in SOD activity or MDA concentration among groups. The PCOS group showed relatively higher SOD activity than the normal control group, whereas the lowest median MDA concentration was observed in Sec200. Increased SOD activity in PCOS may represent a compensatory response to excessive ROS production aimed at maintaining redox balance.(31,32)

The lower median MDA concentration and relatively higher SOD activity observed in Sec200 suggest a more favorable oxidative balance following secretome administration. Extracellular vesicles, growth factors, and antioxidant-associated proteins contained within MSC-derived secretome have been reported to reduce ROS accumulation and attenuate oxidative damage.(29,30) Substantial variability in SOD and MDA measurements was observed among animals, potentially contributing to the absence of statistical significance. Interpretation of these findings is also limited by the lack of biodistribution analysis, which precludes determination of the proportion of secretome-derived bioactive factors reaching the ovary. (33,34) Descriptively, the Sec200 group showed the lowest mean MDA level and the highest mean SOD activity among the treatment groups; however, as these differences were not statistically significant, no optimal dose can be established from the present data.

Disturbances in steroidogenesis resulting from chronic inflammation, oxidative stress, and impaired folliculogenesis contribute to altered estradiol production in PCOS.(6,12) Serum estradiol concentrations did not differ significantly among groups. The PCOS group showed a lower median estradiol concentration than the normal control group, whereas higher median values were observed in secretome treated animals, particularly Sec200. Reduced estradiol production in PCOS is associated with impaired granulosa cell function and follicular arrest. (6,12) Letrozole induced aromatase inhibition further limits the conversion of androgens to estrogens, resulting in disrupted ovarian steroidogenesis.(23,24) Higher estradiol concentrations observed after secretome administration may reflect partial restoration of ovarian function. Secretome derived extracellular vesicles, growth factors, and immunomodulatory mediators have been reported to support tissue repair and granulosa cell activity.(7,28) Modulation of inflammatory and oxidative pathways may also create a more favorable ovarian microenvironment for steroid hormone synthesis.(35,36) Interpretation of these findings is limited by the absence of estrous cycle synchronization before treatment and sample collection. The higher median estradiol concentration observed in Sec200 nevertheless suggests that the 200 µL dose produced the most consistent hormonal profile among the treatment groups.

Chronic inflammation, oxidative stress, and endocrine imbalance collectively contribute to the progression of ovarian dysfunction in PCOS by disrupting follicular development, steroidogenesis, and oocyte competence. (2,5) Elevated TNF-α expression promotes granulosa cell dysfunction and amplifies inflammatory signaling, whereas increased lipid peroxidation further accelerates cellular injury within the ovarian microenvironment. (25,27) Experimental evidence from reproductive disease models has demonstrated that suppression of TNF-α and MDA is associated with improved reproductive outcomes, underscoring the importance of controlling inflammatory and oxidative pathways in ovarian disorders. (37) Preservation of endogenous antioxidant capacity has also been associated with reduced oxidative damage and improved tissue homeostasis, highlighting oxidative stress as a potential therapeutic target.(31,38)

Hormonal disturbances subsequently develop as a consequence of impaired follicular maturation and altered ovarian steroidogenesis. Clinical evidence in women with PCOS has shown that abnormalities in androgen-related hormonal profiles, including serum testosterone, sex hormone-binding globulin, and free androgen index, are closely associated with disease manifestations (39), while impaired granulosa cell function and oxidative stress further compromise aromatase activity and estradiol biosynthesis. (6,33) These findings provide additional biological support for investigating hUC-MSC secretome as a cell-free therapeutic strategy targeting multiple pathological pathways in PCOS rather than a single molecular target.

Several findings might need further confirmation due to several limitations. First, the sample size was small (n=6 per group), which may have limited statistical power to detect between-group differences and may partly explain the lack of statistical significance. Second, animals were allocated to groups by order of arrival rather than by randomization, and outcome assessors were not blinded to group allocation, both of which may have introduced bias. Third, estrous cycle synchronization was not performed before induction, and the cycle stage of normal control rats at sampling was not standardized, which may have

contributed to the variability in estradiol concentrations. (15) Fourth, biodistribution analysis was not performed, precluding determination of the proportion of secretome-derived bioactive factors reaching ovarian tissue; the therapeutic efficacy of secretome-based interventions depends on biodistribution and tissue retention.(10,35,36) Future studies should use randomized and blinded designs, larger sample sizes based on power analysis, estrous cycle synchronization, and biodistribution analysis.

Conclusion

The 200 µL dose showed a favorable trend, with numerically lower ovarian TNF-α expression and MDA levels and higher SOD activity and serum estradiol concentrations compared with untreated PCOS rats, although these differences did not reach statistical significance. These findings suggest a possible modulatory role of hUC-MSC secretome on ovarian function in PCOS and provide a basis for further studies with larger sample sizes to confirm its therapeutic potential.

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Authors Contribution

TAW contributed to study implementation, data acquisition, statistical analysis, and manuscript preparation. RDP oversaw the research project, refined the study design, interpreted the results, and critically revised the manuscript. RAS provided methodological guidance, contributed to data interpretation, and reviewed the manuscript for important intellectual content. All authors participated in the development of the study, approved the final manuscript, and agreed to be accountable for all aspects of the work.

Ethical Statement

The experimental protocol involving animals was reviewed and approved by the Medical and Health Research Ethics Committee, Faculty of Medicine, Public Health and Nursing, Universitas Gadjah Mada, Yogyakarta, Indonesia (Approval No. KE/FK/1803/EC/2025).

Conflict of Interest

The authors have no conflicts of interest to declare.

References

1. Christ JP, Cedars MI. Current guidelines for diagnosing PCOS. N Engl J Med. 2023; 389(16): 1533–45.
2. Kobayashi H, Shigetomi H, Nishio M, Umetani M, Imanaka S, Hashimoto H. Molecular mechanisms underlying reproductive dysfunction in polycystic ovary syndrome. Reprod Sci. 2026; 33(6): 1027–53.
3. Banerjee S, Cooney LG, Stanic AK. Immune dysfunction in polycystic ovary syndrome. Immunohorizons. 2023; 7(5): 323–32.
4. Alam F. Crosstalk between oxidative stress and chronic inflammation in pathogenesis of polycystic ovary syndrome. In: Polycystic Ovary Syndrome: Basic Science to Clinical Advances Across the Lifespan. Elsevier; 2024. p. 43–50.
5. Mancini A, Bruno C, Vergani E, D'Abate C, Giacchi E, Silvestrini A. Oxidative stress and low-grade inflammation in polycystic ovary syndrome: controversies and new insights. Int J Mol Sci. 2021; 22(4): 1667. doi: 10.3390/ijms22041667.
6. Simpson ER, Zhao Y, Agarwal VR, Michael MD, Bulun SE, Hinshelwood MM, *et al.* Aromatase expression in health and disease. Recent Prog Horm Res. 1997; 52: 185–213.
7. Ahangar P, Mills SJ, Smith LE, Gronthos S, Cowin AJ. Mesenchymal stem cell secretome as an emerging cell-free therapeutic strategy. Int J Mol Sci. 2020; 21(19): 7038. doi: 10.3390/ijms21197038.
8. Keshtkar S, Azarpira N, Ghahremani MH. Mesenchymal stem cell-derived extracellular vesicles: Novel frontiers in regenerative medicine. Stem Cell Res Ther. 2018; 9(1): 63. doi: 10.1186/s13287-018-0791-7.
9. Umer A, Khan N, Greene DL, Habiba UE, Shamim S, Khayam AU. The therapeutic potential of human umbilical cord derived mesenchymal stem cells for the treatment of premature ovarian failure. Stem Cell Rev Rep. 2023; 19(3): 651–66.
10. Intan YSN, Soetrisno S, Laqif A, Indarto D, Dirgahayu P, Pamungkasari EP, Putra A. Secretome from human umbilical cord mesenchymal stem cells ameliorates ovarian aging in a murine model. Trends Sci. 2026; 23(10): 13489. doi: 10.48048/tis.2026.13489.
11. Diehl KH, Hull R, Morton D, Pfister R, Rabemampianina Y, Smith D, *et al.* A good practice guide to the administration of substances and removal of blood, including routes and volumes. J Appl Toxicol. 2001; 21(1): 15–23.

12. Kawilarang S, Wiratnaya IGE, Yasa IWPS, Budiana ING. The role of secretome in restoring ovarian function: A systematic review and meta-analysis of in vivo studies in mice with premature ovarian insufficiency. Turk J Obstet Gynecol. 2025; 22(2): 163–9.
13. Hubscher CH, Brooks DL, Johnson JR. A quantitative method for assessing stages of the rat estrous cycle. Biotech Histochem. 2004; 80(2): 79–87.
14. Goldman JM, Murr AS, Cooper RL. The rodent estrous cycle: Characterization and utility in toxicological studies. Birth Defects Res B Dev Reprod Toxicol. 2007; 80(2): 84–97.
15. Ajayi AF, Akhigbe RE. Staging of the estrous cycle and induction of estrus in experimental rodents: an update. Fertil Res Pract. 2020; 6: 5. doi: 10.1186/s40738-020-00074-3.
16. Morsi AA, A Mersal E, Razik H Farrag A, M Abdelmoneim A, M Abdelmenem A, S Salim M. Histomorphological changes in PCOS rat model and contribution of stevia extract. Acta Histochem Cytochem. 2022; 55(1): 9–23.
17. Percie du Sert N, Ahluwalia A, Alam S, Avey MT, Baker M, Browne WJ, *et al.* Reporting animal research: Explanation and elaboration for the ARRIVE guidelines 2.0. PLoS Biol. 2020; 18(7): e3000411. doi: 10.1371/journal.pbio.3000411.
18. American Veterinary Medical Association. AVMA Guidelines for the Euthanasia of Animals: 2020 Edition. Schaumburg (IL): American Veterinary Medical Association; 2020.
19. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2−ΔΔCt method. Methods. 2001; 25(4): 402–8.
20. Elabscience Biotechnology. Superoxide Dismutase (SOD) Assay Kit Instruction Manual. Wuhan: Elabscience Biotechnology; 2026.
21. Elabscience Biotechnology. Malondialdehyde (MDA) Assay Kit Instruction Manual. Wuhan: Elabscience Biotechnology; 2026.
22. Elabscience Biotechnology. Rat Estradiol (E2) ELISA Kit Instruction Manual. Wuhan: Elabscience Biotechnology; 2026.
23. Kafali H, Iriadam M, Ozardali I, Demir N. Letrozole-induced polycystic ovaries in the rat: A new model for cystic ovarian disease. Arch Med Res. 2004; 35(2): 103–8.
24. Bries AE, Webb JL, Vogel B, Carrillo C, Keating AF, Pritchard SK, *et al.* Letrozole-induced polycystic ovary syndrome attenuates cystathionine-β synthase mRNA and protein abundance in the ovaries of female Sprague Dawley rats. J Nutr. 2021; 151(6): 1407–15.
25. Mirseyyed SF, Zavareh S, Nasiri M, Hashemi-Moghaddam H. An experimental study on the oxidative status and inflammatory levels of a rat model of polycystic ovary syndrome induced by letrozole and a new high-fat diet. Int J Fertil Steril. 2023; 18(1): 45–53.
26. Rudnicka E, Kunicki M, Suchta K, Machura P, Grymowicz M, Smolarczyk R. Inflammatory markers in women with polycystic ovary syndrome. Biomed Res Int. 2020; 2020: 4092470. doi: 10.1155/2020/4092470.
27. Spritzer PM. Inflammatory mediators in polycystic ovary syndrome: the case of interleukin-18. Arch Endocrinol Metab. 2022; 66(1): 1–2. doi: 10.20945/2359-3997000000455.
28. Harrell CR, Jankovic MG, Fellabaum C, Volarevic V. Molecular mechanisms responsible for therapeutic potential of mesenchymal stem cell-derived secretome. Cells. 2019; 8(5): 467. doi: 10.3390/cells8050467.
29. Ferreira JR, Teixeira GQ, Santos SG, Barbosa MA, Almeida-Porada G, Gonçalves RM. Mesenchymal stromal cell secretome: Influencing therapeutic potential by cellular pre-conditioning. Front Immunol. 2018; 9: 2837. doi: 10.3389/fimmu.2018.02837.
30. Trigo CM, Rodrigues JS, Camões SP, Solá S, Miranda JP. Mesenchymal stem cell secretome for regenerative medicine: Where do we stand? J Adv Res. 2025; 70: 103–124.
31. Haitham F. Biomarkers of oxidative stress in polycystic ovary disorder. Ann Coll Med Mosul. 2019; 41(2): 112–6. doi:10.33899/mmed.2020.164152.
32. Herrmann IK, Wood MJA, Fuhrmann G. Extracellular vesicles as a next-generation drug delivery platform. Nat Nanotechnol. 2021; 16(7): 748–59.
33. Anwar AA, Abdullah N, Padjalangi AN, Hamid F, Mappeware NA, *et al.* Serum leptin concentration is correlated to insulin resistance in polycystic ovary syndrome (PCOS) patients. Mol Cell Biomed Sci. 2021; 5(2): 93–7.
34. Abdalla MA, Deshmukh H, Atkin S, Sathyapalan T. A review of therapeutic options for managing polycystic ovary syndrome. Ther Adv Endocrinol Metab. 2020; 11: 2042018820938305. doi: 10.1177/2042018820938305.
35. Roszkowski S. Therapeutic potential of mesenchymal stem cell-derived exosomes for regenerative medicine applications. Clin Exp Med. 2024; 24(1): 46. doi: 10.1007/s10238-023-01282-z.
36. Tieu A, Lalu MM, Slobodian M, Gnyra C, Fergusson DA, Montroy J, *et al.* An analysis of mesenchymal stem cell-derived extracellular vesicles for preclinical use. ACS Nano. 2020; 14(8): 9728–43.
37. Brahmana IB, Widjiati, Hendarto H, Widodo MA. Mahkota Dewa (Phaleria macrocarpa) fruit extract decreases malondialdehyde, tumor necrosis factor-α, and caspase-3 levels may improve infertility in endometriosis mice. Indones Biomed J. 2022; 14(3): 287–96.
38. Wardhani SP, Nadliroh KA, Aishaqeena AMF, Sonia FA, Adji AGHS, Hanim IM, *et al.* Ganoderma lucidum polysaccharide peptide reduces oxidative stress and improves renal function in patients with cardiometabolic syndrome. Indones Biomed J. 2020; 12(3): 246–54.
39. Bayuaji H, Nataprawira HMN, Sastramihardja HS. The correlation of serum total testosterone, sex hormone-binding globulin and free androgen index with Athens Insomnia Scale score in women with polycystic ovary syndrome. Indones Biomed J. 2016; 8(3): 177–84.