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TLR4/NF-κB Signaling Contributes to the Inflammation in Ovary and Inguinal Fats of Polycystic Ovary Syndrome

1 **Bo Sun[1]☯, Zimeng Pan[2]☯, Hongying Kuang[3], Xiaoling Feng[3], Lihui Hou[3], Fang Xu[3], Feifei**
2 **Song[4], Miao Sun[3]***

3 [1]Department of Biochemistry and Molecular Medicine, University of California at Davis,
4 Sacramento, USA

5 [2]Department of First Clinical Medical College, Heilongjiang University of Chinese Medicine,
6 Harbin, China

7 [3]Department of Obstetrics and Gynecology, First Affiliated Hospital, Heilongjiang University of
8 Chinese Medicine, Harbin, China

9 [4]Department of Pediatrics, The Fifth Affiliated Hospital of Southern Medical University, Guangzhou,
10 China

11 ☯These authors contributed equally to this work.

12 *** Correspondence:**
13 Miao Sun
14 sunmiao82@163.com

15 **Keywords: polycystic ovary syndrome, inflammation, TLR4/NF-κB signaling, ovary, inguinal**
16 **fats, mechanism, letrozole, different time points.**

17 **Abstract**

18 **Background.** Polycystic ovary syndrome (PCOS) is a complex reproductive endocrine disorder. It
19 has highly heterogeneous clinical manifestations which are characterized by biochemical
20 hyperandrogenemia, obesity, insulin resistance, dyslipidemia, anovulation, and polycystic ovaries.
21 However, the etiologies of PCOS are still unclear. Recently, studies have found that the low-grade
22 inflammation contributed to the occurrence of PCOS, and as a critical biomarker indicated the
23 endocrine disruptions in PCOS. **Objective.** This study is aimed to investigate the processes and
24 mediators of inflammation in contributing to the development of PCOS. **Methods**. Letrozole (LET)
25 induced PCOS rat model was used in this study. Body weight, body temperature, inguinal fats
26 weight, fasting glucose level, ovarian morphology, NF-κB signaling target genes in ovary, and
27 protein expression levels of TLR4 and NF-κB in ovarian and inguinal fats were measured in rats with
28 placebo and LET administrations for 6 and 12 weeks. **Results.** PCOS rats, especially with LET
29 intervention for 12 weeks, had higher body weight, inguinal fats weight and fasting glucose level
30 compared to control group. The protein expression levels of TLR4 and NF-κB in cytoplasm of
31 ovarian and inguinal fats were increased in LET-induced PCOS rats compared to control groups,
32 while NF-κB in nucleus were reduced in PCOS rats. The expressions of ACTB, C3, CXCL3, NQO1
33 and SELP in ovarian were statistically different in PCOS rats induced by LET compared to control
34 groups. **Conclusion.** These findings indicated that stimulating TLR4/NF-κB pathway in inguinal fats
35 and ovary tissues contributed to the increased inflammation in LET-induced PCOS rats, which, in
36 turn, exacerbated the phenotype of PCOS including weight gain, adipose tissue accumulation,
37 hyperglycemia and follicular dysplasia.

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38 **1 Introduction**

39 Polycystic ovary syndrome (PCOS) is the most common endocrine disorder which affects 4%-20%
40 women of reproductive age in the worldwide [1]. It is characterized by biochemical and/or clinical
41 hyperandrogenemia, chronic anovulation, and polycystic ovaries [2]. PCOS usually occurred with
42 other accompany symptoms including insulin resistance, dyslipidemia and abdominal obesity. It
43 contributes to increased risks of developing metabolic syndrome which is a disease covering type 2
44 diabetes, cardiovascular diseases and hypertension [3]. PCOS severely influence the people's health
45 and brought essential social and economic burdens. However, the underlying mechanism is still
46 unclear.

47 Studies have found PCOS patients have higher levels of inflammatory markers (C-reactive protein,
48 interleukin 6 [IL-6], interleukin 18 and tumor necrosis factor-α [TNF-α]) compared with age- and
49 BMI-matched healthy controls [4–6]. Studies suggest that chronic low-grade inflammation is
50 associated with metabolic disorder and ovarian dysfunction in women with PCOS [7–9]. The chronic
51 low-grade inflammation in PCOS women is found to be associated with hypertrophy adipocyte in
52 which the compressed stromal vessels in adipose tissue causes poor blood supply leading to adipose
53 tissue hypoperfusion and, consequently, hypoxia. Then cytokines were secreted and released in local
54 adipose tissue [10]. Interestingly, the insulin resistance, dyslipidemia, and hyperandrogenism might
55 be also in connection with lipid-induced proinflammatory response in PCOS [11]; study also
56 suggests that the adipose tissue as a potential producer of these cytokines (e.g., TNF-α, leptin and
57 resistin) contributing to this inflammation in PCOS [10]. Further, visceral adipose tissue contribute to
58 maintaining this inflammation through regulating the secretions of cytokines and monocyte
59 chemoattractant proteins, as well as recruiting more immune cells [9]. Therefore, chronic
60 inflammation has been increasingly recognized as a critical target in studying the etiologies of PCOS.

61 PCOS can severely affect the follicle quality and oocyte maturation, in which cause infertility in
62 women [12]. Inflammation plays an adverse effect on ovulation which are involved in premature
63 ovarian failure, physiological aging of the ovaries and PCOS [9]. It found that the local inflammatory
64 microenvironment in the ovary lead to abnormal follicular development and ovulation disorders [13].
65 It is also known that the inflammation in circulation plays an adverse effect on oocytes through
66 altering follicle microenvironment, oxidative stress and granulocyte macrophage colony stimulating
67 factors [14]. Therefore, the development of local inflammatory in the ovary as a key in studying
68 PCOS needs more attentions.

69 Up to now, animal PCOS models have been developed efficiently with the characteristic features
70 of PCOS; the most extensively reported is using testosterone, letrozole (LET) and estradiol valerate
71 induced rats and mice model [15]. An animal model which can exhibit all symptoms of PCOS is the
72 most desirable, since PCOS is a heterogeneous disease. LET is a nonsteroidal aromatase inhibitor
73 [16], and can be administrated through oral or subcutaneous [17]. Our previous study indicated that
74 rats with administration of LET subcutaneously for 12 weeks showed both the reproductive and
75 metabolic alterations as PCOS patients have [18]. Although many studies have used LET-induced
76 animal models to study the pathogenesis and pharmacological treatment related to PCOS, most of
77 them focus on the characteristics related to the end induction of the model [19–24], and only a few
78 experiments have been conducted to investigate the pathological mechanisms of LET-induced PCOS
79 model animals at different time points [25,26]. Further, few studies work on investigating the
80 regulation of inflammatory response during the pathogenesis of PCOS.

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81 Therefore, this study intends to investigate the inflammatory processes and mediators that
82 contribute to the commencement and development of PCOS across two different timepoints. By
83 doing so it can broaden our understanding in the association between PCOS and inflammation,
84 provide new direction on preventing and delaying the process of PCOS.

85 2 Materials and Methods

86 Animals.

87 Female Wistar rats were purchased from Experimental Animal Center of University of Heilongjiang
88 Chinese Medicine (Harbin, China). All experimental procedures involving rats were approved by the
89 University of Heilongjiang Chinese Medicine Animal Care and Use Committee and conformed to the
90 Animal Welfare Act 1999. Briefly, female Wistar rats were housed under controlled conditions (21-
91 22°C and 12 h light, 12 h dark cycle). Rats were fed on commercial chow and tap water ad libitum.
92 On age of 21 days, female Wistar rats were randomly assigned to four groups: LET group 1 (LET1)
93 (N=3, 200 μg/day; 60-day continuous-release LET pellets), LET group 2 (LET2) (N=3, 200 μg/day;
94 90-day continuous-release LET pellets), control group 1 (Con1) (N=3, 200 μg/day; 60-day
95 continuous-release placebo pellets, the main ingredient of which is starch), and control group 2
96 (Con2) (N=3, 200 μg/day; 90-day continuous-release placebo pellets). LET (HY-14248,
97 MedChemExpress, USA) and placebo (Innovative Research of America, USA) were implanted with
98 subcutaneous either 60 days or 90 days continuous-release pallets (Innovative Research of America,
99 Sarasota, FL). The 200 μg /day dose was chosen based on previous (18). The flow chart of this study
100 is shown in Figure 1. Food intake, body weight and body temperature were measured every day and
101 calculated weekly from the second week of implantation. Rats were culled and tissues were collected
102 at 6 weeks of LET1 and Con1 and 12 weeks of LET2 and Con12 after pellet implantation,
103 respectively.

104 Fasting Blood Glucose Assays.

105 Rats were fasted overnight, and 1 ml of blood was collected from the tail vein. Blood samples were
106 centrifuged (3000 rpm, 10 min, 4°C) (Remi, C-24 BL, Mumbai, India). Glucose in serum was
107 measured by Glucose Oxidase and Peroxidase (GOD-POD) kit according to the manufacture's
108 instruction.

109 H&E staining and Immunohistochemistry.

110 Rats were culled by isoflurane asphyxiation for tissues collection after 6 and 12 weeks of LET
111 administration. Ovaries and inguinal fats were collected freshly and weighed. One ovary and 0.5 cm
112 length segment of inguinal fats were used for histology and the other ovary and last inguinal fats
113 were stored at -80°C until processing for Western Blot. To observe ovary and fat morphology
114 changes, hematoxylin and eosin (H&E) staining was used. Ovary and inguinal fat pads were fixed in
115 10% neutral buffered formalin for 24h and stored in 70% ethanol before performing H&E staining
116 and immunohistochemistry. For H&E staining, tissues were embedded in paraffine using standard
117 techniques.

118 Ovaries and inguinal fat pads were respectively, serially sectioned at 5 μm and then stained with
119 hematoxylin and eosin. Images were collected using Olympus DP73 microscope. For
120 immunohistochemistry, the protocol was described according to Shaaban´s study (27). Briefly, the
121 ovary and inguinal fat sections were de-waxed, rehydrated, and performed antigen retrieval in 0.1 M

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122 sodium citrate buffer for 10 min in a microwave. Sections were incubated in 3% hydrogen peroxide
123 for 15 min to eliminate endogenous peroxidases. Sections were blocked with goat serum for 15 min
124 at 37 °C followed by incubation overnight at 4 ℃ with primary antibodies Toll-like receptor 4
125 (TLR4) (1: 200, WL00196, wanleibio, China) and nuclear factor-κB (NF-κB) (1: 200, WL01980,
126 wanleibio, China). Following three washes in PBS, sections were incubated with biotin-labeled goat
127 anti-rabbit IgG (H+L) (1: 500, #31460, ThermoFisher, USA) for 30 min at 37 °C. Signals were
128 visualized by reaction with the DAB peroxidase substrate kit (DA1010, Solarbio, China). Images
129 were collected using Olympus DP73 microscope.

130 **Western Bolt.**

131 30 mg ovaries and inguinal fats were respectively used for protein extraction. 40 μg total protein per
132 sample was loaded onto the gel. The methods and procedures were described in previous [28]. The
133 primary antibody we used are TLR4 (1:1000, WL00196; Wanleibio, China) and NF-κB P65 (1:500,
134 WL01980; Wanleibio, China). Goat anti-rabbit IgG-horseradish peroxidase (HRP) (1:5,000,
135 WLA023; Wanleibio, China) was used as secondary antibody. β-actin was used to normalize the
136 expressions of either TLR4 or NF-κB in each sample. The intensities of the protein signals were
137 detected and quantified by Gel-Pro-Analyzer (WD-9413B; Liuyi Biology, China).

138 **qRT-PCR Array Analysis.**

139 92 related genes expression profiles were measured using rat NF-κB Signaling Targets qPCR Array
140 (Supplementary TableS1) according to the manufacturer's protocol (Wcgene Biotech, Shanghai,
141 China). Data was validated using real time quantitative reverse transcription PCR (qRT-PCR). Total
142 RNA (1 μg) was extracted using Trizol and proceed to reverse transcription reactions using the High
143 Capacity cDNA Reverse Transcription kit (Applied Biosystems, Foster City, CA). qRT-PCR was
144 performed on a 7900HT Sequence Detection system (Applied Biosystems) using FastStart Universal
145 SYBR Green Master kit (Roche Diagnostics, Indianapolis, IN) following the manufacturer's
146 instructions. GAPDH was used as a housekeeping gene. Each test was done in triple replication and
147 the $2^{-\Delta\Delta Ct}$ method was used to calculate the expressions of genes. Data were analyzed using Wcgene
148 Biotech software. Genes with fold-changes more than or less than 2.0 were considered to be of
149 biological significance.

150 **Statistical Analysis.**

151 Social sciences (SPSS version 21.0; SPSS, Chicago, IL) and Prism GraphPad (version 6.00,
152 GraphPad Software, La Jolla, CA) were used for testing the statistical difference. Differences
153 between controls and rats exposed to LET were assessed by one-way ANOVA after Dunnett's post
154 hoc test. The differences of body weight, body temperature and food intake between groups were
155 analyzed by repeated-measures ANOVA. Values are mean ± S.E.M. $P < 0.05$ was defined as the
156 threshold of significant.

157 **3 Results**

158 **Body Weight Accumulation and Temperature Changes.**

159 The continuously released pellet containing placebo or LET were implanted subcutaneously at age of
160 21 day. The accumulation of body weight was recorded for 6 weeks and 12 weeks as shown in Figure
161 2A. Significantly increased body weight was observed in rats at 5 and 6 weeks after LET

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162 implantation (Con1 vs LET1, $P<0.05$). In addition, the body weight of LET2 group were increased
163 compared with the Con 2 group during 7-12 weeks, $P<0.05$. Further, the changes of body
164 temperature were fluctuated in the Con1 and Con2 groups, while the body temperature of LET2 rats
165 were lower than Con2 group at the 7th, 8th and 9th week (Con2 vs LET2, $P<0.05$), as shown in
166 Figure 2B.

167 **Inguinal Fats Weight and Fasting Blood Glucose Level.**

168 The weights of inguinal fats tissue in four groups were compared and shown in Figure 3A. The
169 weight of inguinal fats was increased as the treatment going (Con1 vs Con2, $P<0.05$). In addition,
170 the weight of inguinal fats in rats at 6 and 12 weeks of LET implantation was higher than control
171 group at the same period (Con1 vs LET1, $P < 0.05$; Con2 vs LET2, $P < 0.001$), and the inguinal fats
172 weight at 12 weeks of LET implantation was higher than that at 6 weeks ($P < 0.001$). This suggested
173 that the pathological state of PCOS was associated with the increased inguinal fats tissue weight. As
174 the severity of PCOS increased, inguinal fats tissue also gradually got weight.

175 Figure 3B showed the fasting blood glucose level of the four groups. The fasting blood glucose
176 level of rats at 6 and 12 weeks of LET implantation was higher than their corresponding control
177 groups (Con1 vs LET1, $P < 0.001$; Con2 vs LET2, $P < 0.001$). And the fasting blood glucose level at
178 12 weeks of LET implantation was higher than that at 6 weeks ($P < 0.01$). This suggested that the
179 risk of having glucose metabolism disorders was significantly increased in PCOS and became more
180 severe as PCOS disease progresses.

181 **Ovarian Morphology Changes.**

182 More follicles were observed in the ovarian cortex of Con1, Con2 and LET1 groups compared with
183 LET2 group (Figure 4A, B and C). Luteum was clear, cumulus and oocytes were visible, granulosa
184 cell layers were mostly 8 ~ 9 layers and closely arranged, and mature follicles were intact without
185 hyperemia and edema in Con1, Con2 and LET1 groups. The ovary of LET2 had cystic changes, with
186 obvious cystic dilatation of follicles, more atresia, and reduced number of granulosa cell layers and
187 luteal body (Figure 4 D).

188 **The TLR4 and NF-κB Protein Expression in Ovaries and Inguinal Fats.**

189 The abundance of TLR4 and NF-κB protein expressions in ovaries and inguinal fat tissues were
190 measured using immunohistochemistry and western blot. We found that the intensity of TLR4 and
191 NF-κB immunostaining were increased in both ovaries (Figure 5D and Figure 6D) and inguinal fat
192 tissues (Figure 5H and Figure 6H) in LET2. Consistently, western blot showed that the relative
193 expression of TLR4 protein in ovaries and inguinal fat tissues were increased in LET groups
194 compared to control groups (Con1 vs LET1, $P < 0.05$; Con2 vs LET2, $P < 0.05$, Figure 5I). And an
195 increased expression of TLR4 was observed in LET2 compared with LET1 (LET2 vs LET1, $P <$
196 0.05, Figure 5I). Moreover, the protein expression of NF-κB in cytoplasm of ovaries and inguinal fat
197 tissues were also increased in LET groups compared to control groups (Con1 vs LET1, $P < 0.05$;
198 Con2 vs LET2, $P < 0.05$, Figure 6I). A greater increase in NF-κB expression in cytoplasm was also
199 seen in LET2 compared to LET1 group (LET2 vs LET1, $P < 0.05$, Figure 6I). While the expression
200 of NF-κB in nucleus was reduced in LET groups compared to their control groups in ovaries and
201 inguinal tissues (Con1 vs LET1, $P < 0.05$; Con2 vs LET2, $P < 0.05$, Figure 6J). What´s more, there

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202 was a greater decrease in NF-κB expression in nucleus in LET2 compared to LET1 group (LET2 vs
203 LET1, $P < 0.05$, Figure 6J).

204 **The PCR array of NF-κB signaling targets in ovary.**

205 The genes involved in the NF-κB signaling were investigated using qRT-PCR and the results were
206 shown in Supplementary data (TableS2). There were significant differences in the expressions of
207 ACTB, C3, CXCL3, NQO1, and SELP genes between groups. ACTB expression in LET2 was higher
208 than that in LET1 (Figure 7A, $P < 0.05$). The expressions of C3 (Figure 7B) and CXCL3 (Figure 7C)
209 in LET2 were higher than that in Con2 ($P < 0.05$). The expression of NQO1 in LET1 was higher than
210 that in Con1 and LET2 (Figure 7D, $P < 0.05$). The expression of SELP in LET1 was higher than that
211 in Con1 (Figure 7E, $P < 0.05$).

212 ## 4 Discussion

213 PCOS is a complex reproductive and endocrinal disease which the pathogenesis remains unclear. The
214 current treatments are mainly focused on symptomatic approaches rather than curative. Importantly,
215 exploring the pathological mechanisms of PCOS is an urgent to be addressed. Our previous study
216 indicates that the continuous administration of LET, 200μg/d, to female rats for 12 weeks before
217 puberty results in a PCOS model with both the reproductive and metabolic phenotypes [18]. This
218 modeling method has also been used in many PCOS researches [29–31]. Recent studies indicated that
219 increased cytokines were observed promotes in PCOS patients [32,33]. In order to further understand
220 the association between pathological process of PCOS and inflammation, the changes of inflammatory
221 response and metabolic characteristics in PCOS rats were investigate at 6 and 12 weeks after the LET
222 intervention in this study. It was found that the state of high inflammatory status occurred before PCOS
223 was developed, and this inflammation was also accompanied with obesity, accumulation of inguinal
224 fats, hyperglycemia, and abnormal ovarian morphology. Therefore, our study indicated that a pro-
225 inflammatory state might be an important factor in contributing to the development of PCOS.

226 TLR4 belongs to the Toll-like receptor family and expresses in a variety of cells in the body.
227 TLR4 can initiate innate immune responses, modulate adaptive immunity, and defense against
228 pathogens through recognition of pathogen-associated molecular patterns [34]. NF-κB, an important
229 nuclear transcription factor, is a key downstream signal molecule in TLR4 signaling pathway [35], and
230 can initiate the transcription of genes involved in the inflammation and immune responses [36]. In
231 order to investigate the changes of inflammation during the pathogenesis of PCOS, this study examined
232 TLR4 and NF-κB in ovaries and inguinal fat at different timepoints in LET-induced PCOS rats. It is
233 known that NF-κB can bind to inhibitor of κB (IκBα) in the cytoplasm, but once dissociated from IκBα
234 [37,38], allowing NF-κB to enter the nucleus, and NF-κB binds to target genes' promoter regions,
235 thereby triggering the expression of many genes involved in immune and inflammatory responses
236 [39].Therefore, in the present experiment, we examined the expression levels of NF-κB in the nucleus
237 and cytoplasm in inguinal fats and ovarian tissues, respectively. The results revealed that high
238 expression levels of TLR4 and NF-κB in cytoplasm were found in both inguinal fats and ovarian tissues
239 of rats at either 6 or 12 weeks of LET intervention, and the expression levels were higher at 12 weeks
240 than at 6 weeks. However, NF-κB in nucleus expression was low in inguinal fats and ovarian tissues
241 at 6 and 12 weeks of LET intervention and was even lower at 12 weeks than at 6 weeks. Differentially
242 expressed levels of NF-κB in nucleus and cytoplasm level may represent the activation of TLR4/NF-
243 κB signaling pathway and indicate the occurrence of inflammatory responses in inguinal fats and
244 ovarian tissues. At present, more studies have confirmed that some drugs, such as Shaoyao-Gancao

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245 Decoction [40], Astragaloside IV [41] and Soy isoflavones [42], could reduce the inflammatory
246 response in PCOS rats through modulating the NF-κB/TLR4 pathway; the effect is associated with
247 altering gut microbiome structure, regulating secretion of proinflammatory cytokines and enhancing
248 anti-inflammatory capacity. Our results also provide a theoretical basis for future drugs to treat PCOS
249 by modulating the mechanism of TLR4/NF-κB in inguinal fats and ovarian tissues.

250 In PCOS patients, obesity is a common characteristic with an estimated prevalence of 49% and
251 the risk is 2.8-fold higher among women with PCOS than healthy women [43]. As obesity is often
252 linked to chronic low-grade inflammation, inflammation may be a contributing factor to obesity-related
253 complications [44]. In this study, we found that the body weight of the PCOS rats was higher than that
254 of the control group from the 5th weeks of LET intervention until 12th weeks. However, since this
255 experiment only measured the inflammation level in the PCOS rats at 6 and 12 weeks of LET
256 intervention, the relationship between body weight and inflammation changes during the development
257 of PCOS could not be fully determined. But some studies have shown that inflammation was not only
258 associated with obesity, but increased levels of neonatal systemic inflammation was reported to precede
259 the onset of obesity [45–47]. Moreover, in the treatment of obesity, anti-inflammation has been
260 identified as an important strategy in obesity-related metabolic syndromes [48,49]. Therefore, the
261 interactions between obesity and low-grade inflammation during PCOS need to be further studied.

262 Oh *et al.* proposed that women with PCOS showed a "male-like" fat distributions with excessive
263 abdominal (inguinal and visceral) fat accumulation [50]. Adipose tissue is the main source of producing
264 cytokines [51,52]. There was growing evidence indicating obese mice on high fat diets had higher
265 levels of inflammatory cytokines (e.g., TNF-a, interleukin-1β, IL-6) in their livers and adipose tissues
266 [53,54]. Furthermore, hyperglycemia itself is a mediator of inflammation [55], which can also
267 contribute to increasing inflammatory markers including TNF-α and IL-6 [56]. NF-κB can also be
268 activated and stimulated in response to cellular stress including hyperglycemia, obesity and oxidative
269 stress [57]. Recent studies have shown that in obese PCOS subjects, the NF-κB expression was higher
270 than in lean healthy subjects [58]. Also, obesity, especially the abdominal obese phenotype, plays a
271 significant role in activating the NF-κB pathway [59]. In this study, we found both higher inguinal fat
272 and blood glucose in PCOS rats at 6 and 12 weeks of LET intervention. According to another study,
273 excessive inguinal fat was associated with impaired organ function and chronic inflammation in obese
274 individuals [60]. This is also consistent with the high inflammatory response we observed in the
275 inguinal fats of PCOS rats at 6 and 12 weeks of LET intervention. Furthermore, Lionett *et al.* identified
276 that women with PCOS had lower levels of oxidation and mitochondrial respiration in abdominal and
277 gluteal inguinal fats than healthy women [61]. However, more studies are needed to unravel the specific
278 pathological changes in different adipose tissues among PCOS.

279 Although the etiology of PCOS remains unknown, mounting evidence suggests that follicular
280 dysfunction is involved in causing the infertility in women with PCOS [62]. PCOS patients have
281 increased cystic follicles, a thickened thecal cell layer, loose arrangement of granular cells, and reduced
282 corpus luteum; animal models of PCOS also showed similar alterations [62,63]. In this study, follicles
283 with numerous follicular cysts were only present in the ovaries of LET2 group compared with other 3
284 groups. In addition, the LET2 group showed no fluctuation in the body temperature of PCOS rats
285 during 7-8 and 10-12 weeks of LET intervention, which is also an indication of poor ovulation in PCOS
286 rats. Our study indicates that it may be related to the presence of an inflammatory microenvironment
287 in the ovaries. In the ovary, cytokines are secreted by leukocytes, oocytes and follicular cells [64].
288 These cytokines are involved in multiple biology processes including regulating the synthesis of
289 gonadal steroids folliculogenesis, ovarian cells proliferation, and the function of corpus luteum

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[65,66]. The increased inflammation in PCOS ovaries has also been reported to impact follicular growth, and TLR4/NF-κB activation creates an inflammatory environment in the ovary that disrupts ovulation [67]. Interestingly, the development of follicles in the LET1 group showed no different compared with control groups, although there were high expression levels of TLR4 and NF-κB, it may be due to the inflammation intensity and exposure time are not strong and long enough to induce an adverse effect on ovaries, which needs further research.

NF-κB plays a critical role in the initiation and resolution of inflammation [68] and can been activated and translocate into the nucleus to upregulate transcription of several inflammatory genes [69,70]. We showed, for the first time, that five differentially expressed NF-κB signaling target genes in ovarian tissues in LET-induced PCOS rat at two different timepoints, specifically, ACTB, C3, CXCL3, NQO1 and SELP. So far, only a few studies have explored their pathologic mechanisms in PCOS. Huang team performed cDNA microarray and qRT-PCR analysis of cumulus cells isolated from PCOS patients, and their results suggested that CXCL3 was related to oocyte nuclear maturation in PCOS patients [71,72]. In order to determine the candidate genes of PCOS, Shen et al. performed comprehensive analysis using GSE345269 microarray date which consisted of 7 granulosa cell samples of PCOS patients and 3 healthy normal granulosa cell samples [73]; it was found that ACTB were upregulated in PCOS patients but the difference was not statistically significant. The complement factor C3 is a pivotal component of inflammation; studies found an association of serum C3 and C-reactive protein (CRP) with insulin resistance in PCOS subjects [74,75]. Studies also identified that NQO1 protein expression was significantly increased in the endometrium of women with PCOS and this change was similar to those found in patients with endometrial cancer [76]. Further functional experiments will be needed to investigate the role of these genes in PCOS, which is also the main focus of our future experiments.

There are some limitations in this study. Samples can be collected and observed at more time points during LET intervention, such as 4 weeks and 8 weeks. On the other hand, we could analyze the inflammatory status in different tissues, such as liver, visceral fats and hypothalamus, and more comprehensively observations of the inflammatory processes in PCOS are needed to have a better understanding of how inflammation contributes to the development of PCOS.

5 Conclusions

This study, for the first time, investigate the associations of inflammation status and PCOS characteristics across two different timepoints. Activation of the TLR4/NF-κB pathway in inguinal fats and ovarian tissues persisted during the formation of LET-induced PCOS in rats. Moreover, ACTB, C3, CXCL3, NQO1 and SELP may be key regulatory target genes in ovarian tissue. Also, the high inflammatory state in PCOS may induce or exacerbate the phenotypic features of PCOS such as hyperglycemia, weight gain, adipose tissue accumulation and follicular dysplasia. Therefore, improving inflammation may play a critical role in preventing or attenuating PCOS. The regulation of the TLR4/NF-κB pathway could be new target in the prevention and treatment of PCOS.

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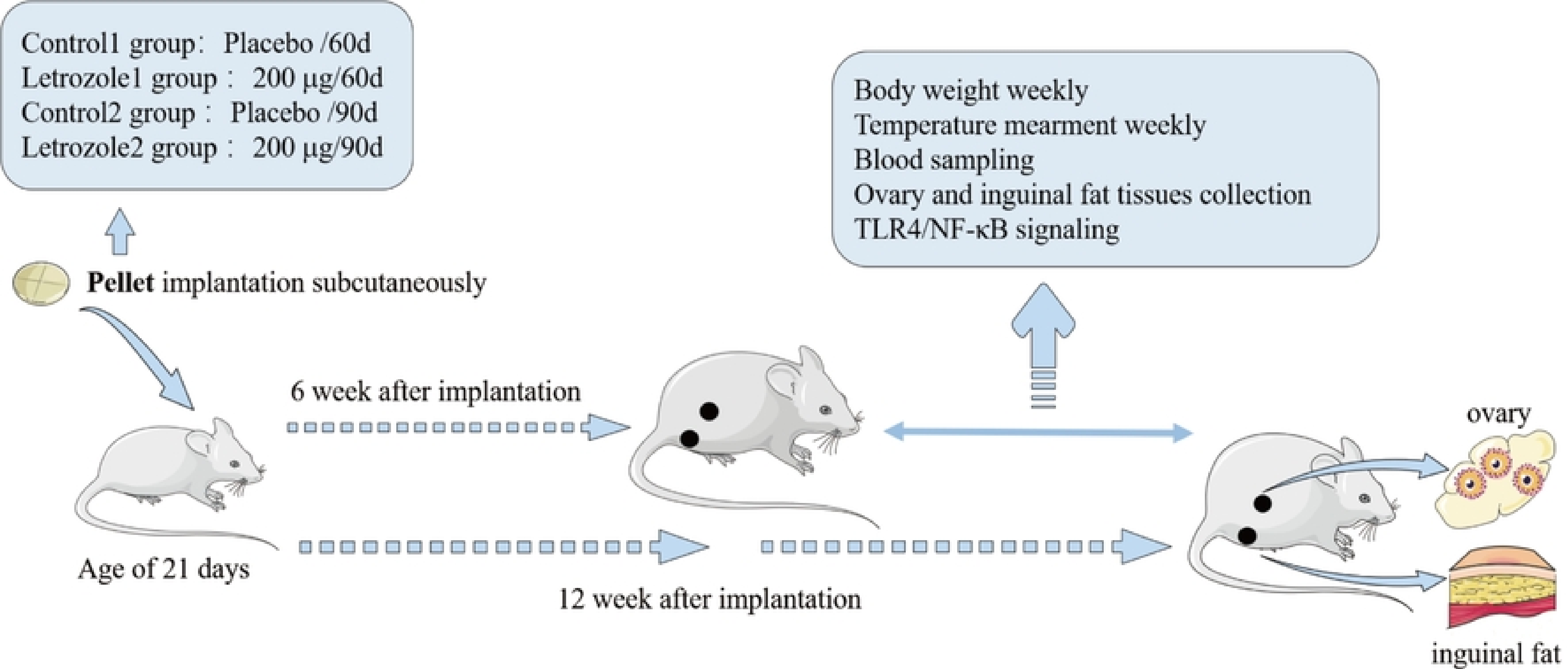

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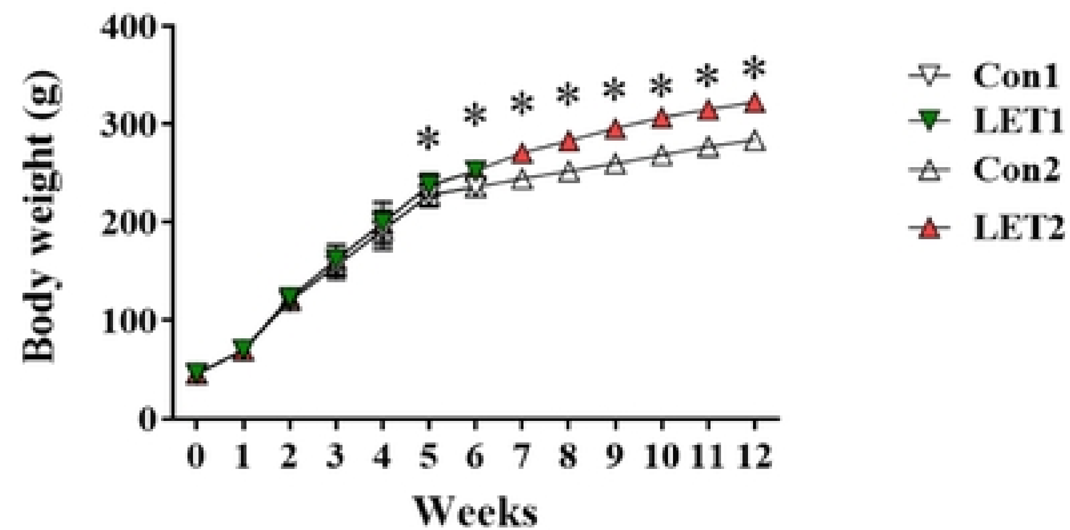

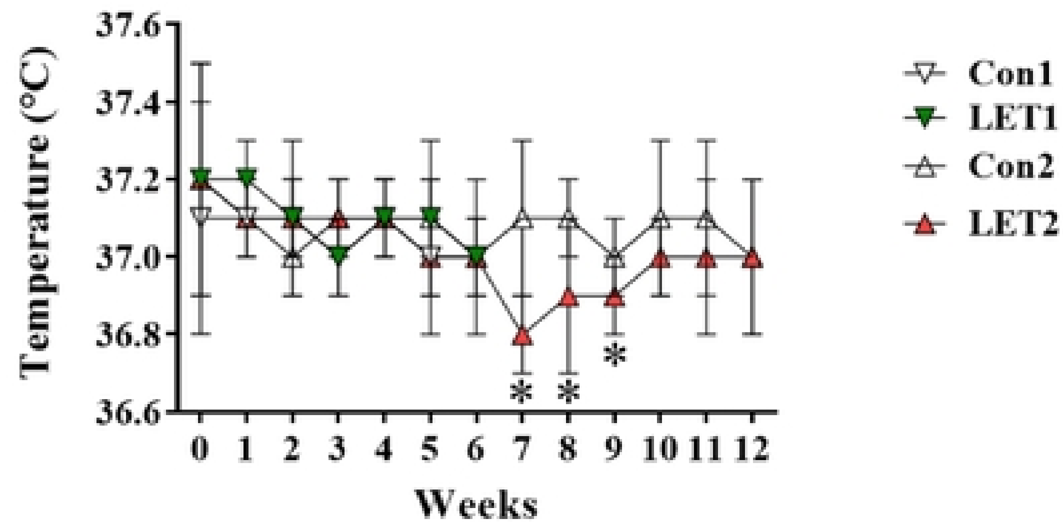

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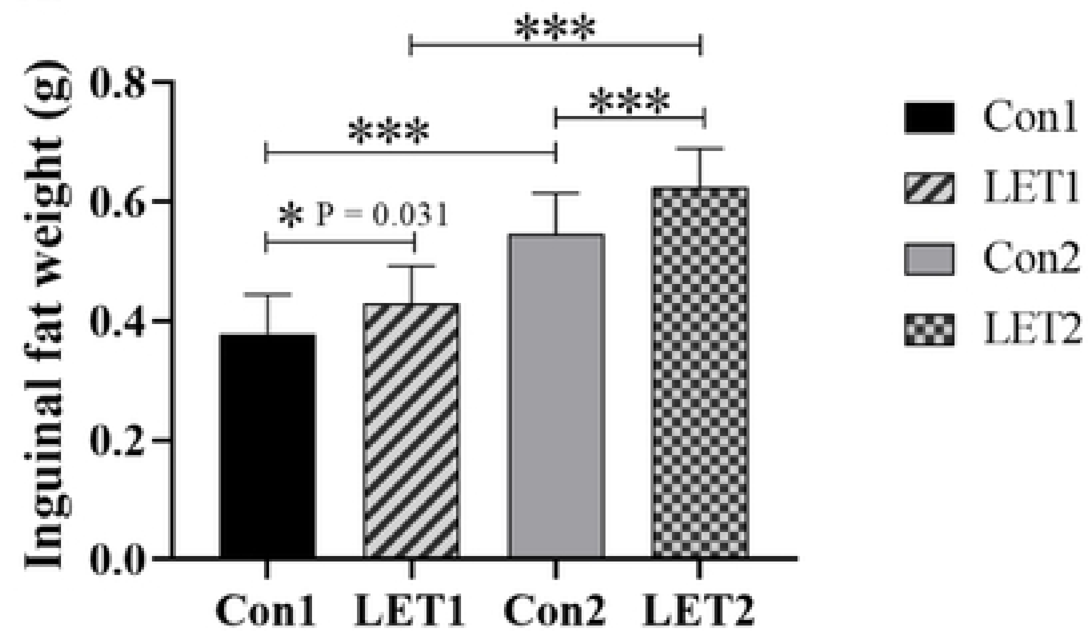


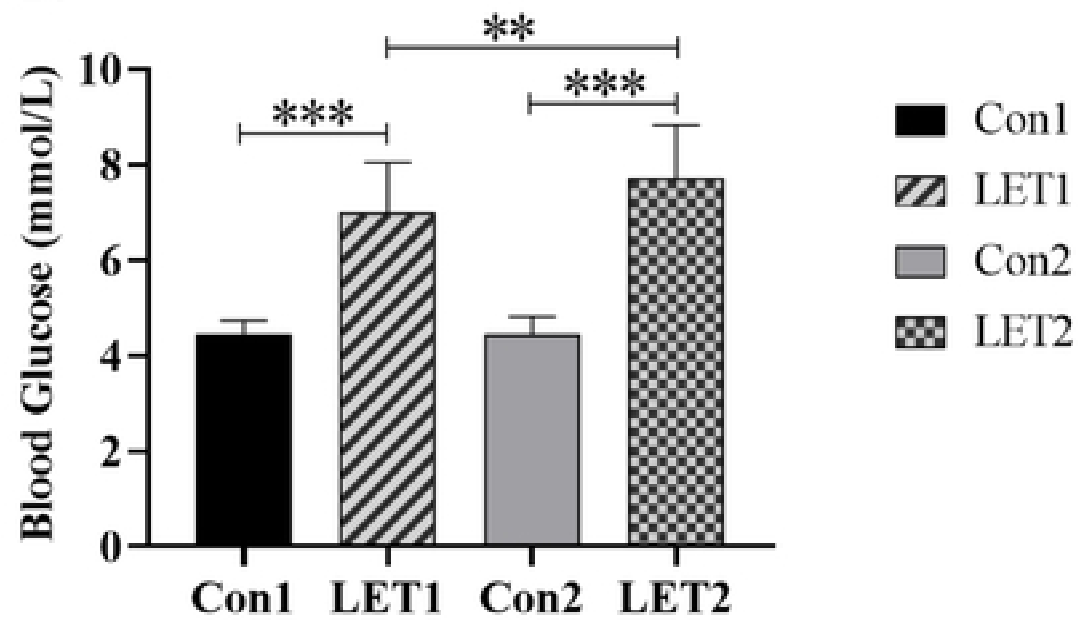
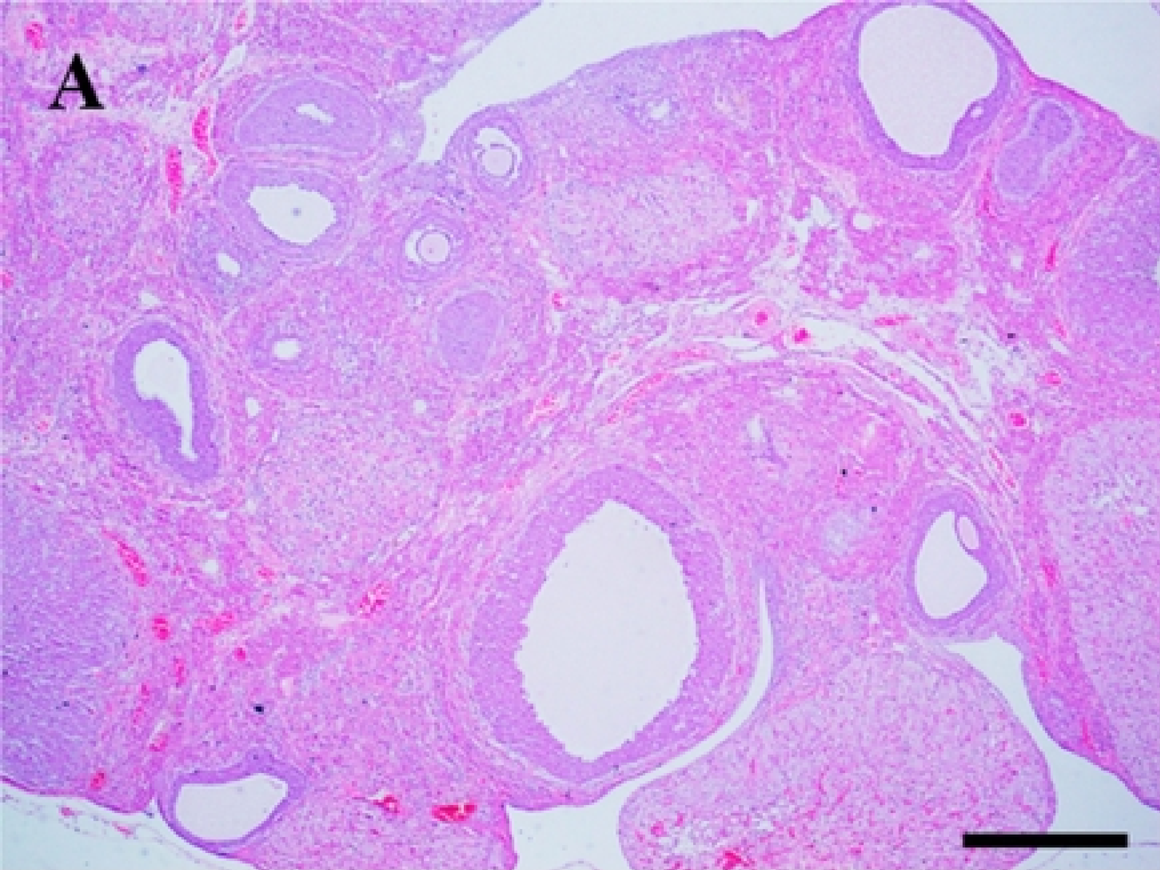
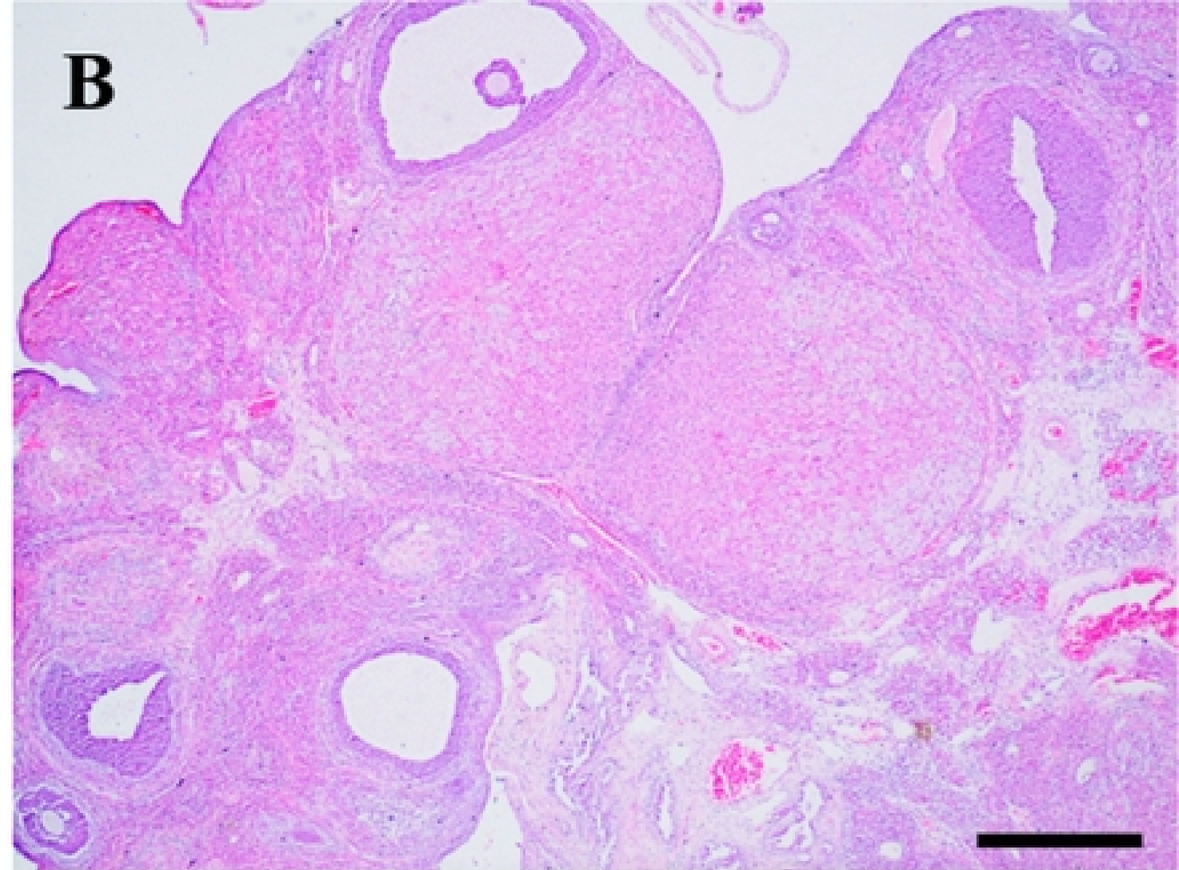
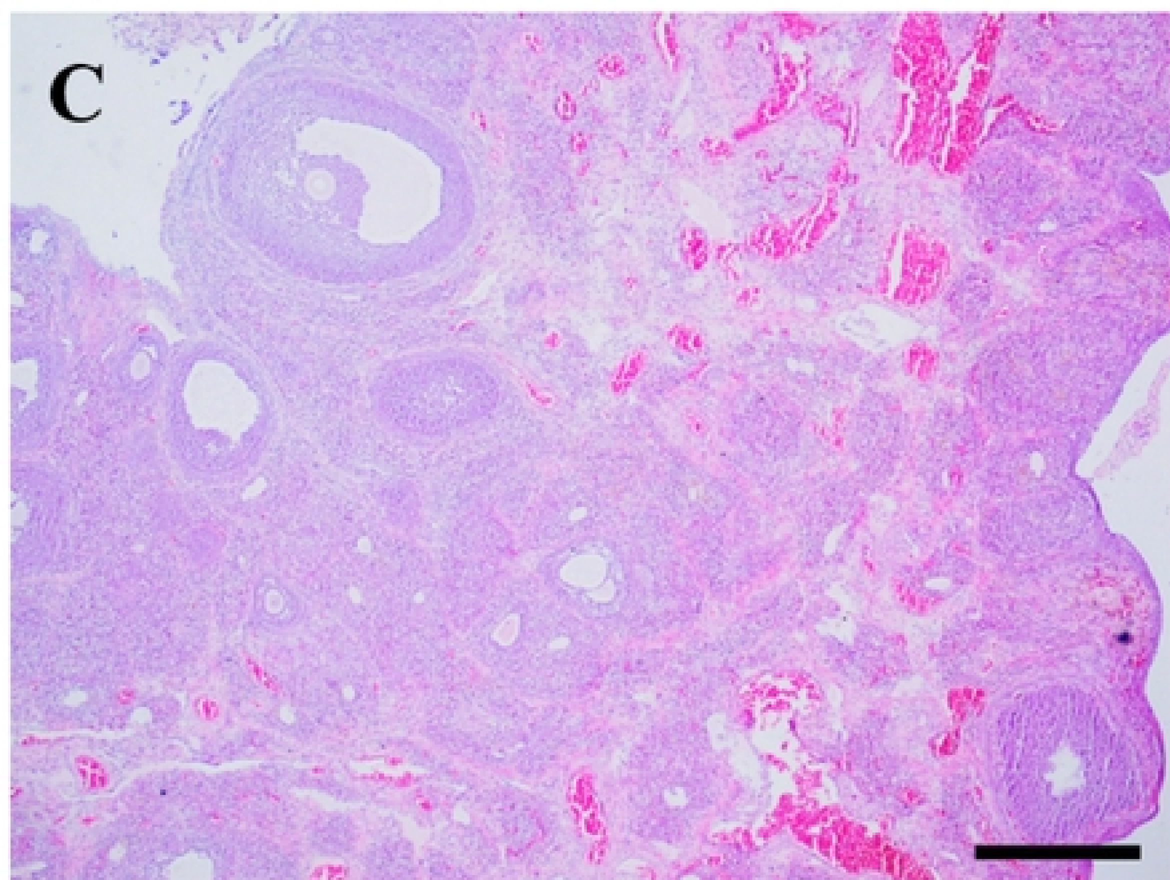


Figure3







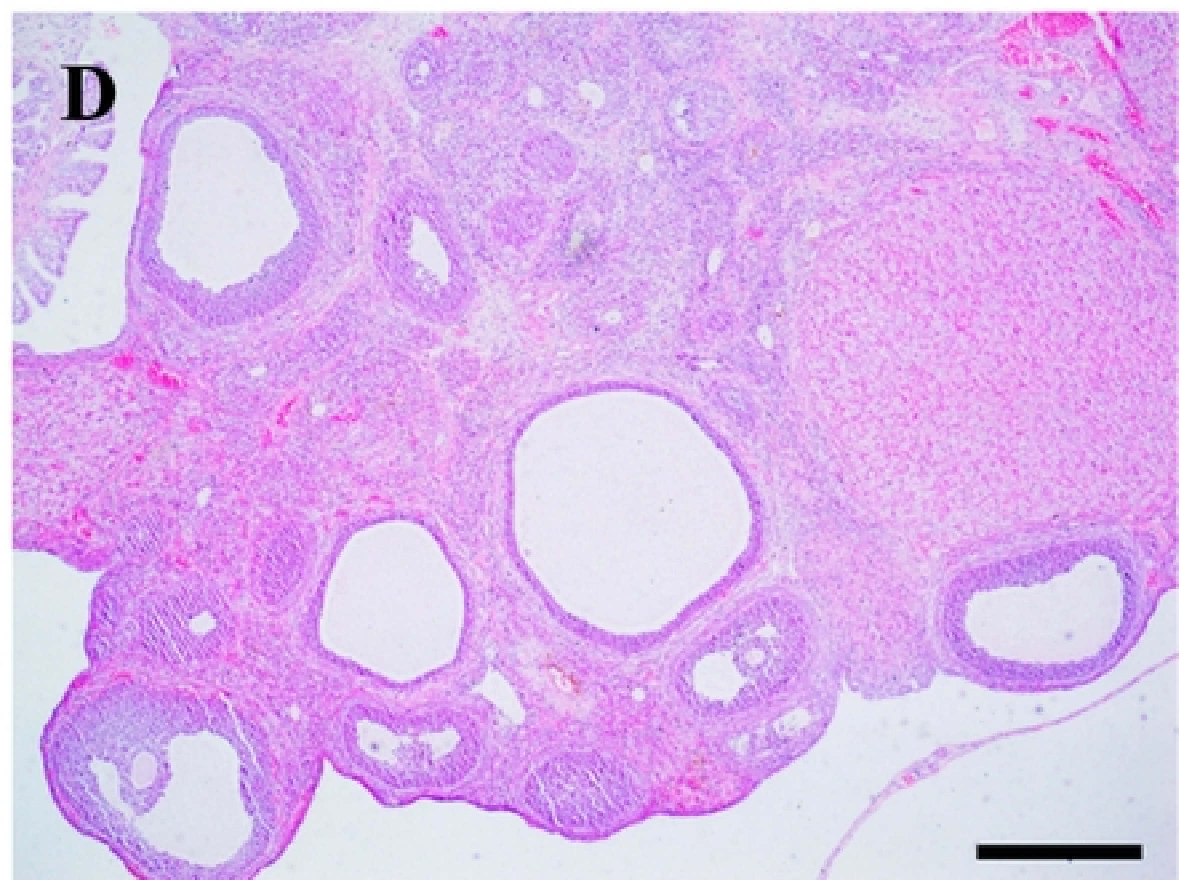


Figure4

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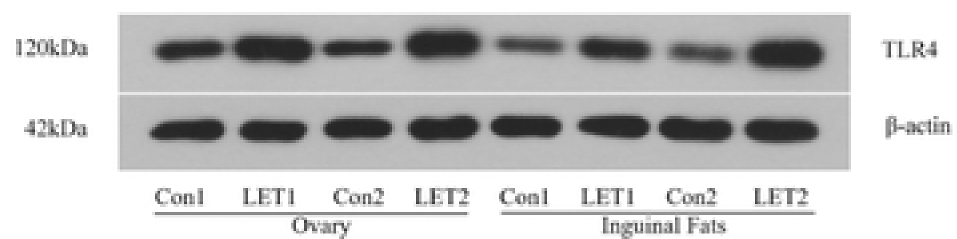


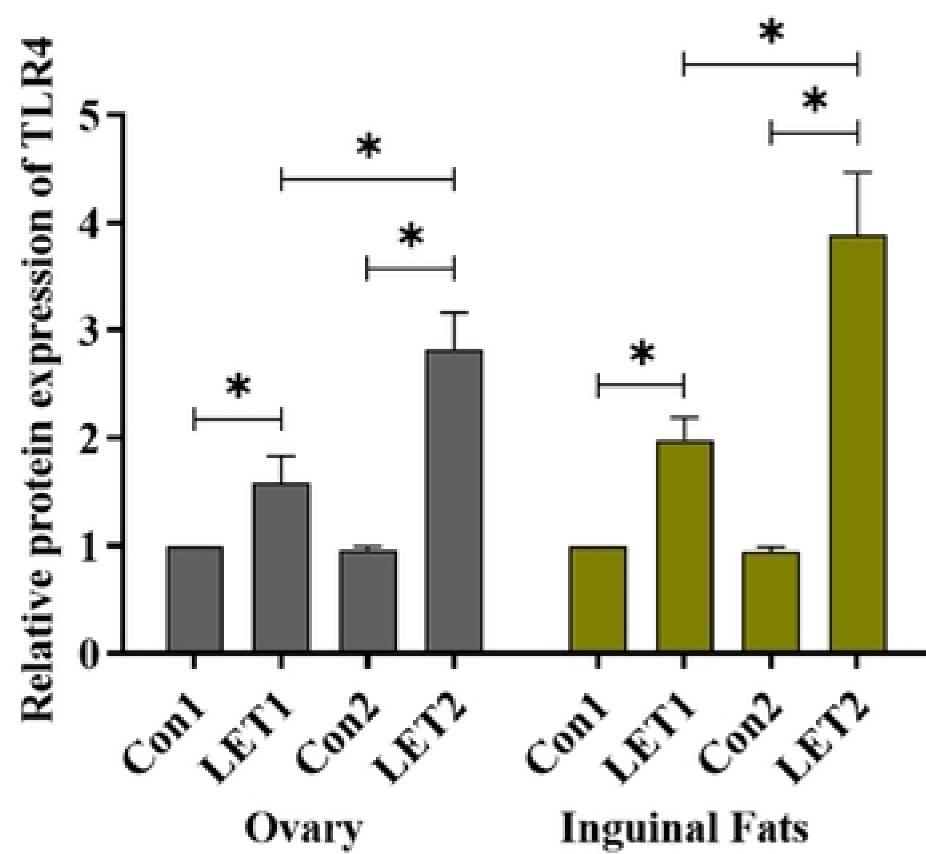
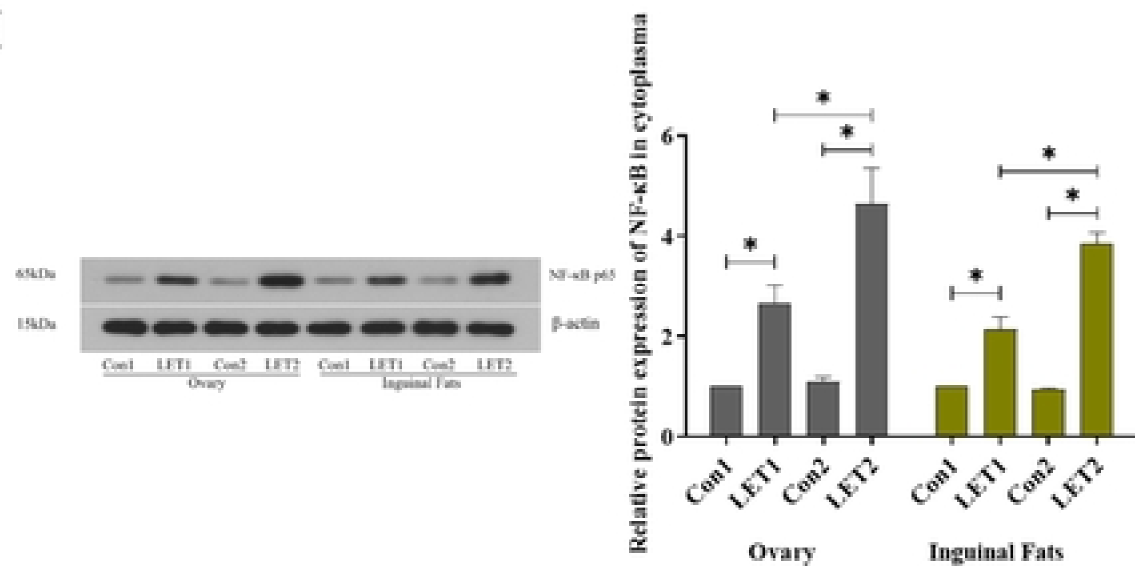


Figure5

A B E F
C D G H



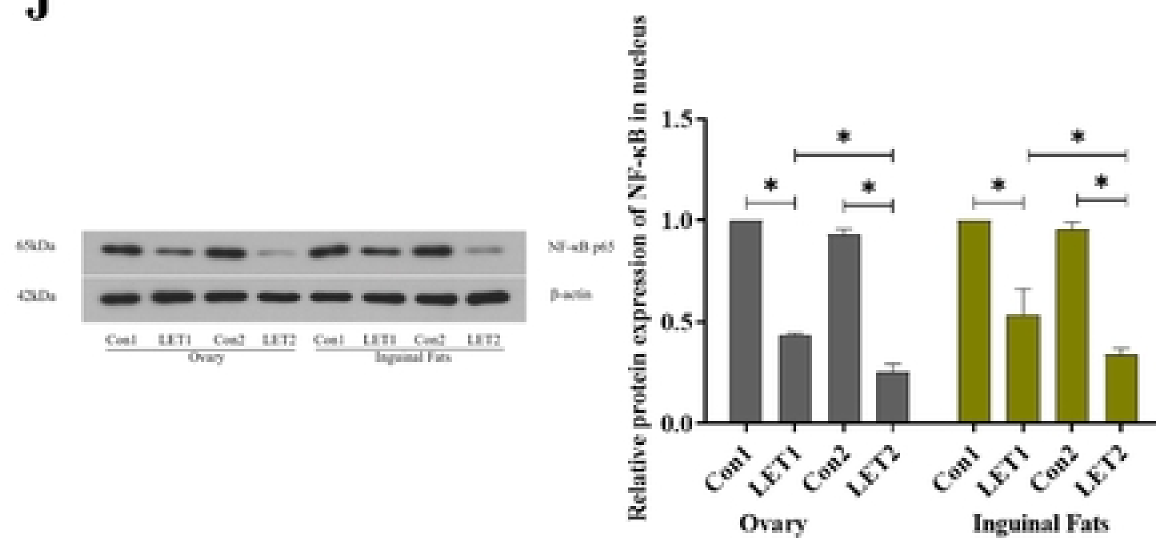
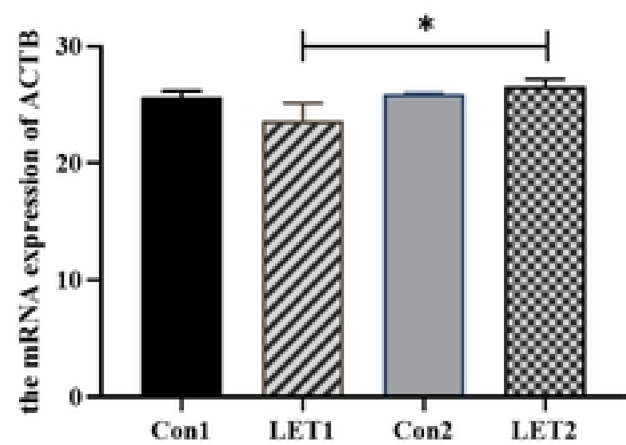
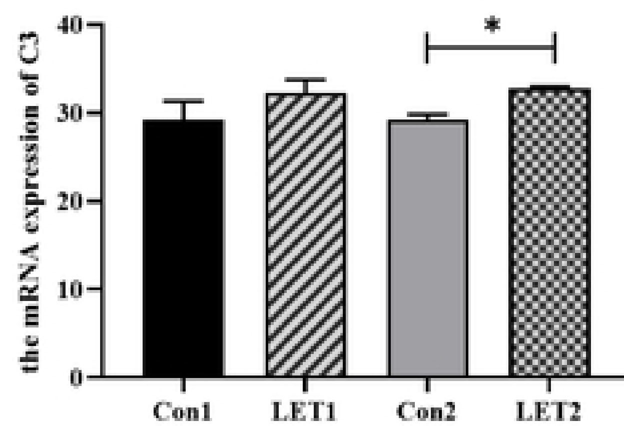
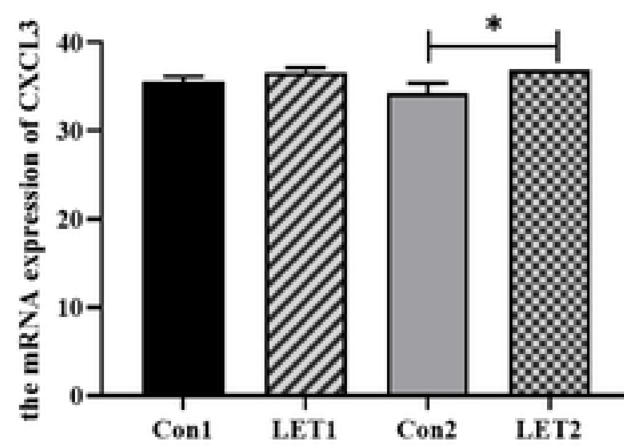
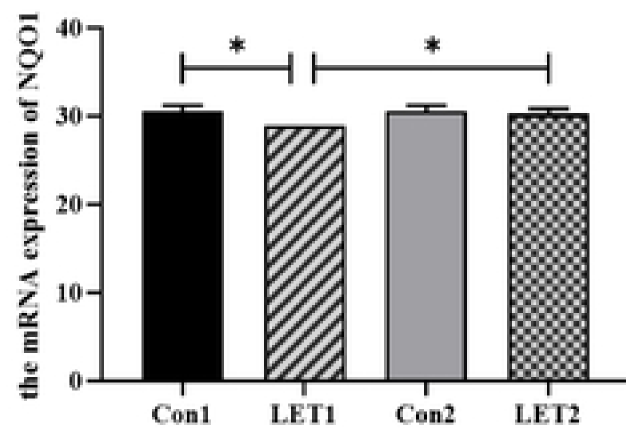


Figure6









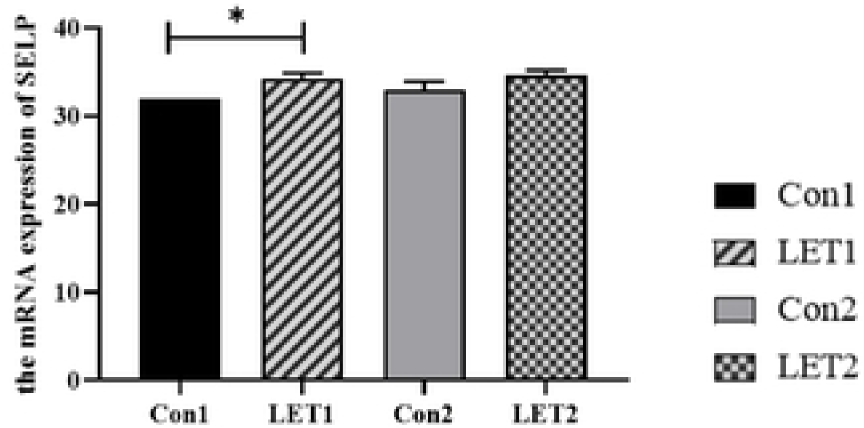


Figure7