



Long-Term Effects of Neonatal LPS and Caspase -1 İnhibitor Administration on Gonadotropin Levels and Testicular Histology in Rats

Sıçanlarda Neonatal LPS ve Kaspaz-1 İnhibitörü Uygulamasının Gonadotropin Düzeyi ve Testis Histolojisi Üzerine Uzun Vadeli Etkleri

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Abstract

Aim: Endotoxins (lipopolysaccharides, LPS) negatively affect reproduction by decreasing gonadotropin levels in the acute period. The aim of this study is to investigate the long-term effects of re-injection of endotoxin in adulthood on gonadotropin release and testicular tissue in male rats injected with endotoxin with IL-1 beta inhibitor (Q-Vd-OPh; caspase-1 inhibitor, CI) in the neonatal period.

Material and Methods: On postnatal day 7, male rat pups were injected once with either Salin, LPS (50µg/kg), LPS (50µg/kg)+CI (1mg/kg). On postnatal 10 months, these rats were either injected with saline or LPS (50 µg/kg). 10 days after these injections, serum samples and testicular tissues were taken and the experiment was terminated.

Results: Respectively, control, Salin+LPS, LPS+Salin; LPS+LPS; LPS(CI)+Salin and LPS(CI)+LPS groups were 18.8±1.6; 11.3±1.2; 23.4±2.0; 22.2±1.2 ve 23.6±1.4 ng/ml for serum LH concentrations (p<0,05); while 33.4±2.5; 29.6±1.4; 32.1,6±2.0 28.7 0.8 and 35.5±3.3 ng/ml for FSH concentrations (p>0,05). Seminiferous tubule damage was observed in LPS groups (p<0,05).

Conclusion: This research demonstrates (1) neonatal LPS injections do not negatively affect gonadotropin release in the long term, (2) IL-1 β plays a critical role in the programming of reproduction, especially in terms of testicular histology.

Keywords: neonatal LPS; LH; FSH; seminiferous tubules; caspase-1 inhibitor

Öz

Amaç: Endotoksinler (lipopolisakaritler, LPS) akut dönemde gonadotropin seviyelerini düşürerek üremeyi negatif yönde etkilemektedir. Bu çalışmanın amacı, neonatal dönemde endotoksin ile birlikte IL-1 beta inhibitörü (kaspaz-1 inhibitörü, CI) enjekte edilen erkek sıçanlara erişkinlik döneminde tekrar endotoksin enjekte edilmesinin gonadotropin salınımı ve testis dokusu üzerine uzun vadeli etkilerini araştırmaktır.

Materyal ve Metod: Erkek sıçan yavrularına neonatal 7. günde Salin, LPS veya LPS+kaspaz-1 inhibitörü (CI) intraperitoneal olarak enjekte edildi. Daha sonra 10. ayda her grup kendi içinde iki ayrı gruba ayrılarak yarısına salin diğer yarısına da aynı dozda LPS enjeksiyonu yapıldı. Bu enjeksiyonlardan 10 gün sonra serum numuneleri ve testis dokuları alınarak deney sonlandırıldı.

Bulgular: Sırasıyla kontrol, Salin+LPS, LPS+Salin; LPS+LPS; LPS(CI)+Salin ve LPS(CI)+LPS grupların için serum LH konsantrasyonları 18.8±1.6; 11.3±1.2; 23.4±2.0; 22.2±1.2 ve 23.6±1.4 ng/ml (p<0,05) iken FSH konsantrasyonları ise 33.4±2.5; 29.6±1.4; 32.1,6±2.0 28.7 0.8 ve 35.5±3.3 ng/ml (p>0,05) olarak tespit edildi. LPS gruplarında seminifer tübül hasarı görüldü (p<0,05).

Sonuç: Bu araştırma göstermektedir ki (1) neonatal LPS enjeksiyonları uzun vadede gonadotropin salınımını olumsuz yönde etkilememektedir, (2) IL-1 β, özellikle testis histolojisi açısından üremenin programlanmasında kritik bir rol oynamaktadır.

Anahtar Kelimeler: Neonatal LPS; LH; FSH; seminifer tübülleri; kaspaz-1 inhibitörü

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INTRODUCTION

It is well known that stressful stimuli, as infection or administration of endotoxin, can have profound negative effect on the activity of reproduction functions (1). Early life may shift the functional of bodily systems, and this contribute to later life health has been defined to as perinatal programming (2). Research investigating this fact has demonstrated long-term changes in immune, metabolic (2, 3), neuroendocrine function (4, 5) and behaviour (6-8). It has been well to determined that the activity of hypothalamo-pituitary-gonadal (HPG) axis is disturbed by immunological stresses. LPS injection suppresses the activity of a GnRH pulse generator (9, 10), affects estrous cycle (9), delays puberty (11-13). Interleukin-1 β has pivotal roles in the LPS induced suppression of the HPG axis (9, 14, 15). Recently, a broad spectrum caspase inhibitor has been developed in which a carboxy terminal phenoxy group is conjugated to the amino acids valine and aspartate (Q-VD-OPh). The administration of QVD-OPH, a caspase inhibitor (CI), causes a decrease in IL-1 α and IL-1 β (16).

In the present study, we examined the effects of neonatal and prepubertal LPS administration on response testicular

histology, serum LH and serum FSH levels. In addition, we determined this effects of neonatal LPS administration role of interleukin-1 β inhibition with caspase-1 inhibitor.

MATERIAL AND METHODS

Animals and Experiment Protocols

Pregnant Wistar-Albino rats (INUDEHUM, Turkey) were housed under controlled conditions (12L:12D; 22 \pm 2 °C) and supplied with ad libitum food and water. This study was approved Animal Research Ethics Committee of Inonu University Medical Faculty (Protocol # 2010/54). On pnd 7, the pups were administered either saline, or 50 g/kg LPS (*Escherichia coli* O111:B4, Sigma L-2630), or 50 g/kg LPS plus 1 mg/kg Q-Vd-OPh (Biovision, California, USA) by intraperitoneal (i.p.) injection. C1i (50 L) was injected 60 min after LPS injection (see Figure 1). The timing of C1i administration in relation to the timing of the LPS injection and this dose were determined from the literature (17). At pnd 7, the control group received both the vehicle for Q-Vd-OPh (i.e., DMSO). At pnd 30, each group was further subdivided into 2 groups, and one of these 2 groups received sterile physiologic saline solution (50 L) and the other received LPS (50 g/kg, 50 L).

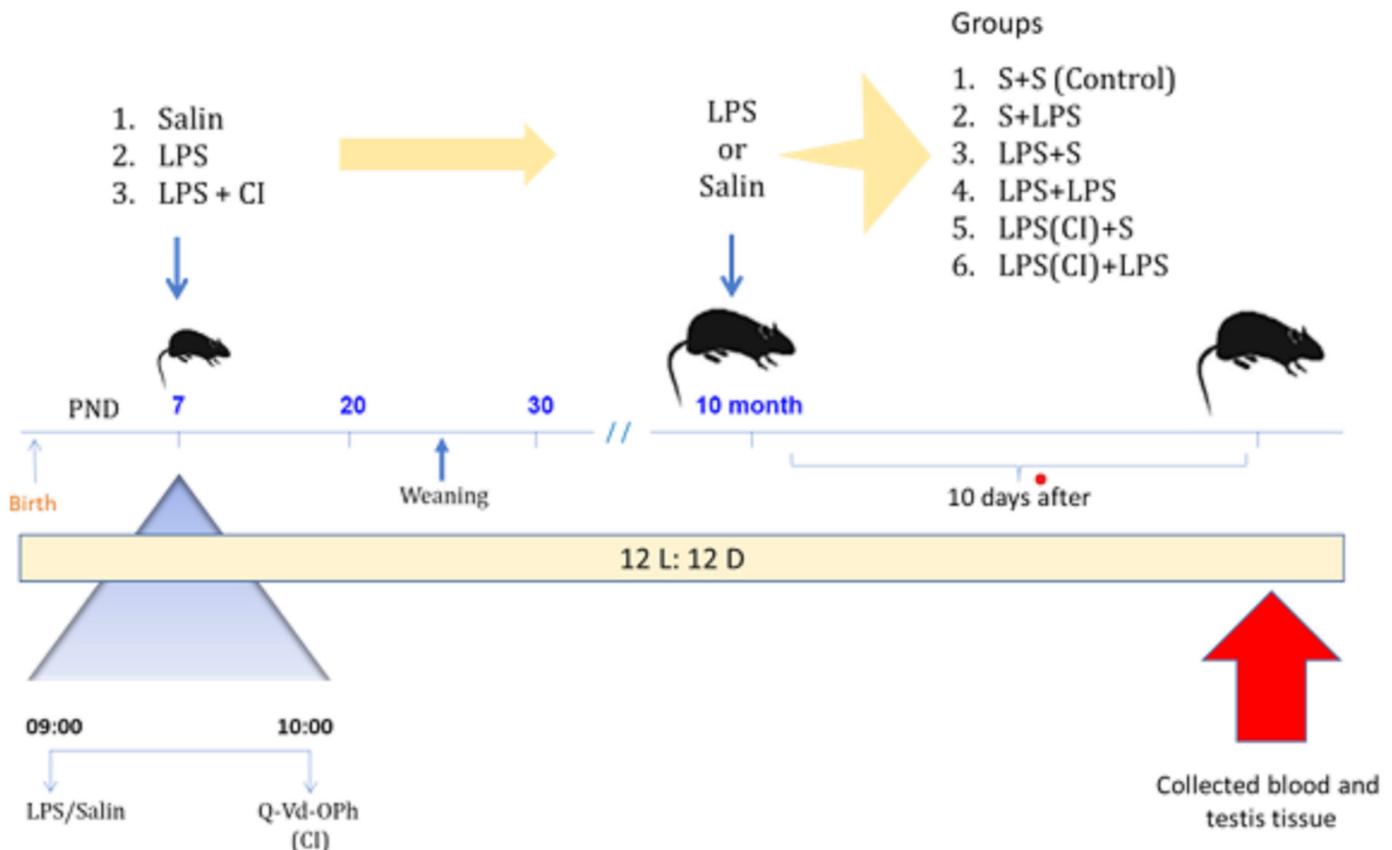


Figure 1. Experimental protocol

Histopathologic Examination

Testis tissue was fixed in 10 % formol and was embedded in paraffin. Sections of tissue were cut at 5 μ m, mounted on slides, stained with hematoxylin-eosin (H-E).

Semiquantitative Evaluation

The diameter and germinative cell layer thickness of the seminiferous tubule (ST) from twenty different areas of each testis were measured using a Leica Q Win Plus Image Analysis System (Leica Micros Imaging Solutions Ltd, Cambridge, United Kingdom) at 10X. Histopathological evaluations were performed according to Sayim F method except minor revision (18). Histological changes were detected by counting 100 tubules in slides stained with H-E. One hundred tubules per animal were examined and classified as normal, sloughing, spermatogenic arrest and germ-cell degeneration based on the degree of seminiferous tubule damage.

The tubules with sloughing were those that showed disrupted cell association. Tubules with anormal shaped cells were classified as tubules with germ cell degeneration. Tubules with interrupted spermatogenic cells at various stage of mitotic phase were classified as tubules with spermatogenic arrest.

LH and FSH enzymeimmunoassays

LH and FSH were analyzed according to Pappa et al. (19) with some modifications. Briefly, 96-well immunoplates (Nunc, Roskilde, Denmark) were coated with rat LH or rat FSH. Serum samples or standards were preincubated with primary antibodies and were then transferred into coated plates for competition with antigens on the solid phase. Plates were washed and the secondary antibody conjugated to streptavidin peroxidase was added into each well and color was developed by using tetramethylbenzidine as the substrate. Plates were read at 450 nm using a plate reader (Biotek, Synergy HT, USA). Rat LH, rat FSH and primary antibodies (rabbit anti-rat LH and rabbit anti-rat FSH) were obtained from Dr. A.F. Parlow (NIDDK, NIH, USA) and secondary antibodies (goat anti-rabbit IgG) conjugated to streptavidin peroxidase was purchased from Sigma (Sigma-Aldrich, Taufkirchen, Germany). Sensitivity of the assays was 1 ng/ml for LH and 2 ng/ml for FSH. Inter- and intra-assay coefficients of variations were below 8 % for both LH and FSH.

Statistical analysis

SPSS 25.0 program was used for statistical analysis. The results were compared with Kruskal-Wallis variance analysis. Where differences among the groups were detected, group means were compared using the Conover test. Values of $p < 0.05$ were considered significant. All results were expressed as means \pm standard error (SE).

RESULTS

There was no significant difference between the groups in terms of FSH levels. LH levels were found to be statistically lower in the Saline + LPS group compared to the control.

The data of LH and FSH findings are presented in Table.3.

Morphological damage ranged from none (control) to slight LPS(CI)+S to moderate S+LPS and LPS+S and to severe LPS+LPS and LPS(CI)+LPS. In control group, the seminiferous tubules were intact and germ cells organized in concentric layers (Figure 2). Histopathological observation showed that LPS administration caused testicular damage characterized by sloughing, spermatogenic arrest and degenerate germ cell. Numerous round germ cells which detected from spermatogenic layers were observed in the some seminiferous lumen in these groups (Figure 3A, 3B and 3C) Also seminiferous tubules containing arrested spermatogenic cells at various stage of division and degenerative changes in germinal cells were observed in LPS groups (Figure 4A, 4B and 4C). The most obvious damage was found in LPS+LPS group compare with LPS-S and S-LPS groups. The most affected group between the single dose LPS groups was detected as LPS-S group. ($p=0.009$). On the other hand, the number of affected seminiferous tubules in the LPS(CI)+S group was significantly lower according to LPS+S and S+LPS groups ($p < 0.05$).

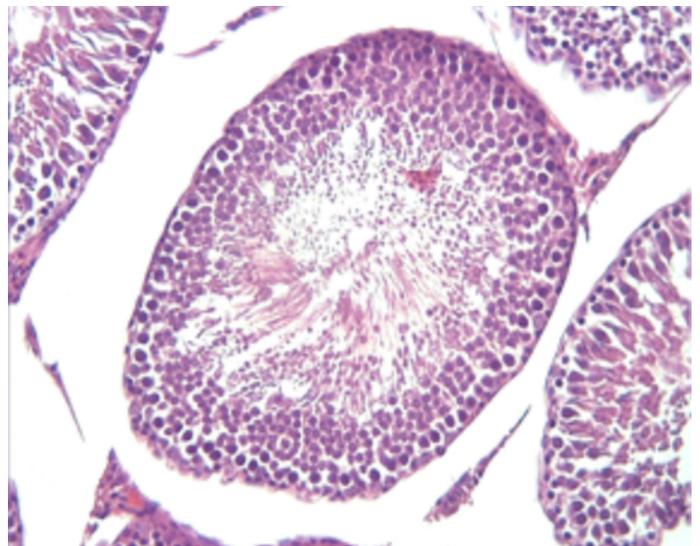


Figure 2. Control; Normal histological view of seminiferous tubules. H-E X66

In LPS(CI)+S group, seminiferous tubules were nearly similar to the control group except disintegration of spermatogenic layer observed in some of the tubules (Figure 5A).

Ci treatment was not found effective on given two dose LPS group. Although intact seminiferous tubular was recognized as increased in LPS(CI)+LPS group, the lesions did not completely ameliorate. Disordered testicular epithelium, degenerative changes in germinal cells and arrested spermatogenic cells were still present in this group (Figure 5B).

Moreover decreased mean seminiferous tubule diameter and decreased germinal cell layer thickness

were prominent in group given two dose LPS group. CI administration was not improved the decreased seminiferous tubule diameter and germinal cell layer. The results of the histopathological classification of the

seminiferous tubules, diameters seminiferous tubules (DST) and germinal cell layer thickness (GCLT) were given Table 1 and Table 2.

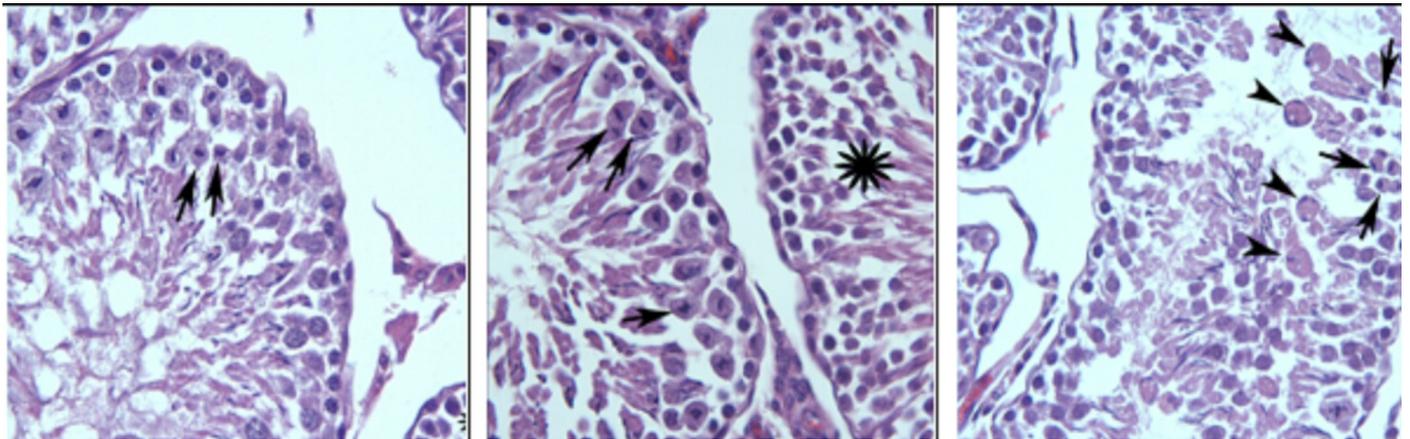


Figure 3. (A) S+LPS; disorder of testicular epithelium is seen in the tubule of top (B) LPS+S; notice the accumulation of immature germ cells in the lumen (C) LPS+LPS large number of immature germ cells in the lumen are present due to disruption of cell-cell contacts. H-E X66

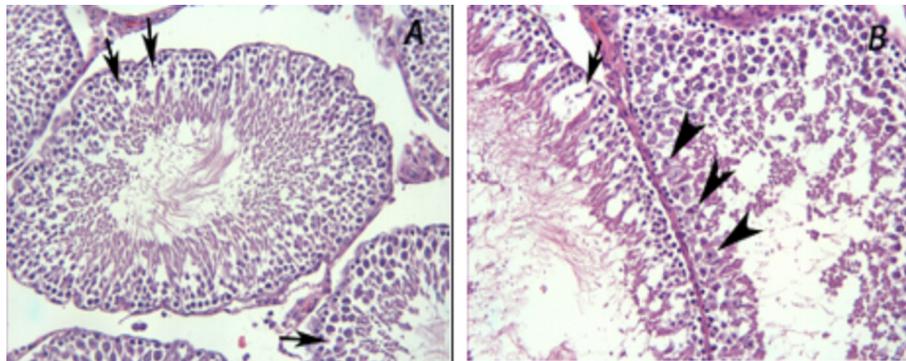


Figure 4. (A) S+LPS and (B) LPS+S; spermatogenic arrest (arrows) is observed in the tubule of left but the other tubules (stars) show nearly normal histologic appearance; (C) LPS+LPS; Degenerate spermatogenic cells (head arrows) are visible in seminiferous tubule. H-E X132

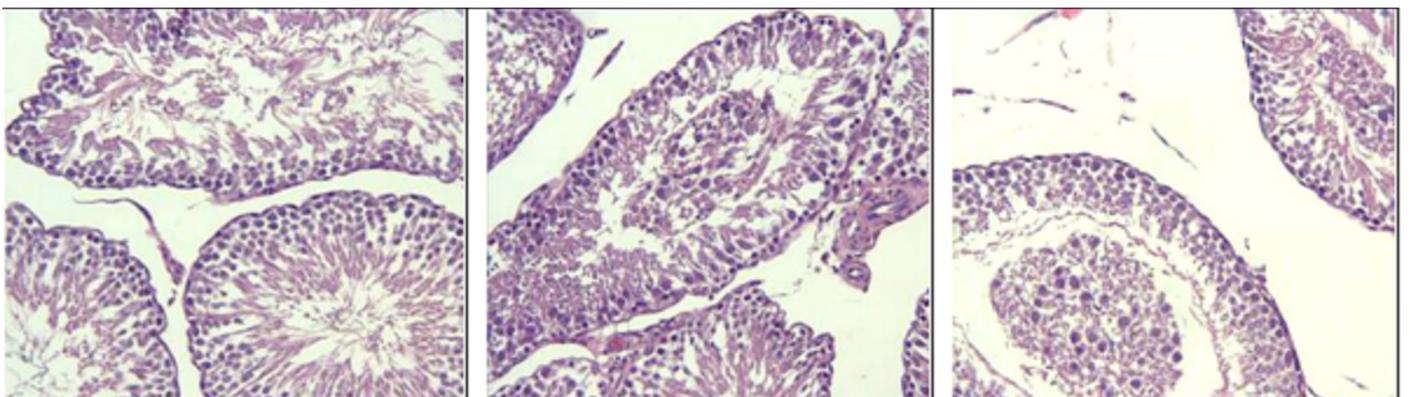


Figure 5. (A) LPS(CI)+S; testicular histology revealed normal except the disintegration of spermatogenic layer (arrows) of some tubules (B) LPS(CI)+LPS; Disordered testicular epithelium (arrow) and spermatogenic arrest (head arrows) are still evident. H-E X 66

Table 1. Percentage (%) of histopathological classification of the seminiferous tubules in the testis

	Control	S+LPS	LPS+S	LPS+LPS	LPS(CI)+S	LPS(CI)+LPS
Intact	99.0 ± 0.3	77.6 ± 0.7 ^a	69.2 ± 0.3 ^a	49.4 ± 1.8 ^{ab}	90.8 ± 0.5 ^{ac}	57.0 ± 0.6 ^a
Sloughing	0.0 ± 0.0	7.6 ± 0.8	14.2 ± 1.1	15.0 ± 1.7	1.8 ± 0.3 ^b	21.6 ± 0.5
SA	0.0 ± 0.0	10.4 ± 0.7	10.4 ± 0.8	27.0 ± 0.8 ^c	6.8 ± 1.0	12.4 ± 0.9 ^d
GCD	1.0 ± 0.4	4.4 ± 0.5	6.2 ± 0.5	8.6 ± 0.5	0.8 ± 0.3	9.0 ± 0.3

SA: Spermatogenic arrest; GCD: Germ-cell degeneration

^aSignificantly decreased when compared with control group (p<0.05).

^bSignificantly decreased when compared with S-LPS and LPS-S groups (p<0.05).

^cSignificantly increased when compared with S-LPS and LPS-S groups (p<0.05).

^dSignificantly decreased when compared with LPS-LPS group (p<0.05).

Table 2. Diameters seminiferous tubules (DST) and germinal cell layer thickness (GCLT)

	Control	S+LPS	LPS+S	LPS+LPS	LPS(CI)+S	LPS(CI)+LPS
DST	634,4 ± 6,4	642,9 ± 6,0	605,5 ± 7,0 ^a	590,6 ± 6,8 ^a	595,0 ± 6,6 ^a	589,6 ± 5,8 ^a
GCLT	100,6 ± 1,6	94,6 ± 1,6 ^a	90,3 ± 1,4 ^a	88,1 ± 1,5 ^a	82,2 ± 1,1 ^a	76,4 ± 0,9 ^a

^aSignificantly decreased when compared with control group (p<0.05).

Table 3. Serum LH and FSH concentrations of the groups

	Control	S+LPS	LPS+S	LPS+LPS	LPS(CI)+S	LPS(CI)+LPS
LH	18.8 ± 1.6	11.3 ± 1.2 ^a	23.4 ± 2.0	22.3 ± 1.5	22.2 ± 1.2	23.6 ± 1.4
FSH	33.4 ± 2.5	29.6 ± 1.4	32.1 ± 2.0	35.1 ± 2.6	28.7 ± 0.8	35.5 ± 3.3

^aSignificantly decreased when compared with control group (p<0.05).

DISCUSSION

Immune stress can cause reproductive dysfunction. Some hypothalamic factors such as pro-inflammatory cytokines play pivotal roles in reproductive disorders under immune stress conditions. According to the data we obtained from our study, the lowest LH level was seen in the S+LPS group, while the lowest FSH level was found in the LPS(CI)+S group. It was determined that the effect of LPS on gonadotropin production in the neonatal period was lower. LPS-induced changes does not alter the effects of LPS on the serum levels of LH or testosterone, in adult male rats (20). Another study reported that the reduction in sexual behavior induced by neonatal stress was not associated to androgen levels in male rats (21). Munkhzaya et al reported that

immune stress did not suppress LH secretion in the early neonatal period in rats (22). According to the literature information, it is seen that a single LPS injection in the neonatal period does not affect androgen release in the long term. It was determined that the LH and FSH levels obtained in the present study were negatively affected by the second injection in the postnatal period rather than the neonatal period. In addition, it was determined that caspase inhibitor had no permanent effect on androgen levels.

In our study, it was determined that LPS administration caused testicular damage and the most damage was seen in the groups receiving double dose LPS. The seminiferous tubule diameters and the thickness of the germinal cell layer of the groups receiving double-dose

LPS decreased. The effect of the caspase inhibitor on this increase was not observed. In the neonatal period, the level of seminiferous tubules affected in the group in which caspase inhibitor was applied with LPS was similar to that of the control. Therefore, the group least affected by LPS was the LPS(CI) group. LPS+S was the most affected group in the single dose LPS groups. As a result, it has been observed that the caspase inhibitor has a protective role in the groups receiving a single dose of LPS, but it is not effective in preventing the degenerative effects of the second LPS on the testis in the later periods. In a study investigating the effect of LPS on spermatogenesis in adult mice at different times, it was reported that harmful effects were observed after 60 days on spermatogenesis (23). It has been reported that LPS-induced cytokine activation causes pathogenic damage in testicular tissue (24). Bacterial infection and inflammation of the testis impairs fertility, yet an understanding of inflammatory responses of the testis is incomplete (25).

According to our findings, neonatal LPS administration does not affect gonadotropin release in the long term. In addition, it is thought that IL1-beta secreted during immune challenge may have negative effects on testicular histology.

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Conflict of Interest: The authors declare that they have no competing interest.

Acknowledgments: The authors declare that there is no conflict of interest associated with this study.

Ethical approval: Animal Research Ethics Committee of Inonu University Medical Faculty (Protocol # 2010/54).

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