Jurnal Kesehatan Gigi 12 Nomor 1 (2025) 71 -76

Jurnal Kesehatan Gigi

p-ISSN: 2407-0866
e-ISSN: 2621-3664

http://ejournal.poltekkes-smg.ac.id/ojs/index.php/jkg/index

Examining the Impact of *Porphyromonas gingivalis* Infection on Ovarian Health: A Reduction in Primordial Follicles in a Female Periodontitis Model

Zahara Meilawaty[1] Argha Sirna Galau[2] Sabania Dinar Permatasari[2] Sari Setyaningsih[1] Pudji Astuti[1] Ernie Maduratna Setiawatie[3] Hendy Hendarto[4] Agustin Wulan Suci Dharmayanti[1]

[1]*Department of Biomedical Sciences and Oral Maxillofacial Pathology, Faculty of Dentistry, University of Jember, Jember, Indonesia*
[2]*Faculty of Dentistry, University of Jember, Jember, Indonesia*
[3]*Department of Periodontology, Faculty of Dental Medicine, Airlangga University, Surabaya, Indonesia*
[4]*Department of Obstetrics and Gynecology, Faculty of Medicine, Airlangga University, Surabaya, Indonesia*

Corresponding author: Agustin Wulan Suci Dharmayanti
Email: agustinwulan.fkg@unej.ac.id

ABSTRACT

Periodontitis is a chronic inflammation of the periodontal tissues caused by periodontal pathogens, such as *Porphyromonas gingivalis* (*P. gingivalis*). *P. gingivalis* possesses several virulence factors that enable it to invade and spread through the bloodstream to other organs, including the ovaries. It triggers inflammation, which may impact the number of primordial follicles. The aim of this study was to assess the effects of *P. gingivalis* infection on the number of primordial follicles in the ovaries. This study was a laboratory experiment with a post-test control group design. The rats were divided into control and periodontitis groups. The periodontitis group consisted of rats induced with *P. gingivalis* in their sulcus gingiva of the first mandible molar every three days for 19 days. Therefore, control groups were without *P. gingivalis* induction. Seven days after the last induction of *P. gingivalis*, the rats were euthanized, and their ovarian tissues were collected for histological preparation. The primordial follicles were counted under 400x magnification. This study showed that the periodontitis group had a significantly lower number of primordial follicles (2.2 ± 2.61) compared to the control group (12.4 ± 2.41) ($p<0.05$). The study concludes that *P. gingivalis* infection led to a reduction in the number of ovarian primordial follicles in periodontitis models. This study underlined the importance of maintaining periodontal health not only for oral health, but also to prevent negative impacts on women's reproductive health.
Keyword: Primordial follicle; Ovaries; Periodontitis; *P. gingivalis*

Introduction

Periodontitis is a chronic inflammation of the periodontal tissues. This condition is characterized by red gingiva, a soft consistency, shiny surfaces, the loss of stippling, and rounded margins, often accompanied by gingival recession [1]. In advanced stages, periodontitis leads to alveolar bone resorption, resulting in tooth mobility and eventual loss [2].

Periodontitis is an oral disease that should not be misjudges due to its prevalence. In 2021, it was reported that approximately 1 billion people worldwide experienced severe periodontitis [3]. The prevalence is notably higher in men (around 57%) compared to women (approximately 39%). This discrepancy are attributed to several factors, including a higher rate of smoking and a generally lower inclination among men to take preventive measures [4]. However, the occurrence of periodontitis in women is particularly concerning, as hormonal fluctuations associated with the menstrual cycle impact the body's hormonal conditions more than in men. This hormonal

instability may enhance the pathogenicity of periodontitis in women [5],[6].

The most effective management of periodontitis focuses on plaque control, as plaque serves as the primary cause of the condition. This biofilm harbors various periodontal pathogens, including *Porphyromonas gingivalis* (*P. gingivalis*), *Prevotella intermedia*, *Actinobacillus actinomycetemcomitans*, *Fusobacterium nucleatum*, and *Tannerella forsythia* [7]. Numerous studies have identified *P. gingivalis* as the predominant bacteria in the gingival sulcus of patients with periodontitis [8]. *P. gingivalis* is particularly notable as a unique periodontal pathogen due to its ability to evade the host's immune response. It possesses several potent virulence factors, including fimbriae, gingipains, and lipopolysaccharide (LPS), which contribute to inflammation, tissue damage, and the pathogen's invasion into other tissues via the bloodstream [9]. This may lead to bacteremia, systemic inflammation, and the translocation of *P. gingivalis* to various organs, including the female reproductive system [10]. Additionally, hormonal fluctuations throughout the female reproductive cycle can influence plaque control and complicate the treatment of periodontitis [11].

There is concern that *P. gingivalis* may enter and disrupt ovarian function through the bloodstream, resulting in potential disorders within the female reproductive system. Several studies detected *P. gingivalis* in the placentas of pregnant women with periodontitis [12],[13]. However, these studies primarily indicate a link between *P. gingivalis* and premature birth, leaving unexplored its potential effects on other aspects of women's reproductive health, such as the folliculogenesis process.

Several studies demonstrated that *E. coli* LPS impacts folliculogenesis by disrupting the primordial follicle reserve in the ovaries [14],[15]. Both *P. gingivalis* and *E. coli* possess similar LPS as a virulence factor. *E. coli* LPS is generally considered more potent, capable of triggering septic shock, while *P. gingivalis* LPS is associated primarily with chronic inflammation [16]. Nevertheless, this does not exclude the possibility that *P. gingivalis* LPS may also influence folliculogenesis. Dharmayanti et al. indicated that induction by *P. gingivalis* may lead to infertility, as evidenced by an increase in the number of atretic follicles in a periodontitis mouse model [17],[18]. However, the effect of *P. gingivalis* induction on primordial follicles remains unclear. The presence of a sufficient number of primordial follicles is crucial for a woman's fertility, as these follicles serve as the precursors to the subsequent growth and development phases leading up to fertilization. This study aimed to differentiate the number of primordial follicles in the ovaries following infection with P. gingivalis.

Methods

This study was an experimental laboratory with a post-test only control group design. Approval for the study was granted by the Medicine and Health Research Ethics Commission at the Faculty of Medicine, Universitas Airlangga, under reference number 275/EC/KEPK/FKUA/2023. The subjects of the study were female white rats (Rattus norvegicus) of the Sprague-Dawley strain, aged 6-7 weeks, and weighing between 120-170 grams, all in the estrous phase and in good health.

The rats were acclimatized for 14 days to adapt to their environment and diet before any treatment was administered. They were provided food and water ad libitum. The rats were divided into two groups: a control group and a periodontitis group, with each group comprising five rats. Sample sizes for each group were calculated using Lemeshow's sample size formula. The control group received no injection of *P. gingivalis*, while the treatment group was injected with P. gingivalis.

Preparation of *P. gingivalis* Suspension

The preparation involves creating a 10 ml liquid media using 0.37 grams of BHI-B, 1 µl of vitamin K, 5 µl of hemin, and 50 µl of yeast extract. An ose of *P. gingivalis* from BHI-A agar media is introduced into the BHI-B. The *P. gingivalis* suspension was then incubated for two 24-hour periods, after which a concentration of 2.10^7 CFU was achieved by adding additional liquid media [17].

Preparation of Periodontitis Model

The periodontitis model was established by inducing *P. gingivalis* in rats. The induction occured at the mesio-buccal and mesio-lingual gingival sulcus of the right and left lower first molars, using *P. gingivalis* suspension volume of 0.05 ml per area. Injections were administered with a tuberculin syringe (#30 gauge) every three days for 19 days. The presence of periodontitis was determined after seven *P. gingivalis* inductions, indicated by signs of redness, grade 3 tooth mobility, and bone resorption (radiographic) [17].

Euthanasia and Ovaries Removing

Euthanasia was performed by administering a lethal dose of ketamine/xylazine. This procedure took place on the 7th day following the last induction of *P. gingivalis* (the 26th day overall since the initial induction). In contrast, the control group was euthanized on the 26th day as well. To remove the ovarian organs, abdominal surgery was conducted after the rats were anesthetized with lethal doses. The obtained ovarian tissues were rinsed with a 0.9% physiological saline solution and subsequently placed in a 4% paraformaldehyde fixation solution for 24 hours. Following this, the tissues were transferred to a 70% alcohol solution as a preservation step (Wahyuni et al., 2019). The ovarian tissues were then processed and stained using Hematoxylin and Eosin (HE). Observations were made under a light microscope at magnifications of 400x across 10 fields of view [18]. The focus of these observations was to quantify the number of primordial follicles. The data collected were analyzed using the Independent Sample T-Test ($P<0.05$) to assess the significance of differences between the periodontitis and control groups.

Results and Discussion

Table 1. Number of Primordial Follicle (N=10)

Group	N	Primordial Follicle	P value
CG	5	12.4 ± 2.41	0.000
PG	5	2.6 ± 2.61	

Data presented mean and standard deviation; Data were analyzed using independent t-test ($p<0.05$); N, number of animal models used in this study; CG, ovaries of control group; PG, ovaries of periodontitis group

Table 1 shows the difference in the number of primordial follicles in the control and periodontitis groups. The number of primordial follicles in the periodontitis group was significantly different from the number of primordial follicles in the control group ($p<0.05$), where the number of primordial follicles in the periodontitis group (2.6±2.61) was less than in the control group (12.4±2.41).

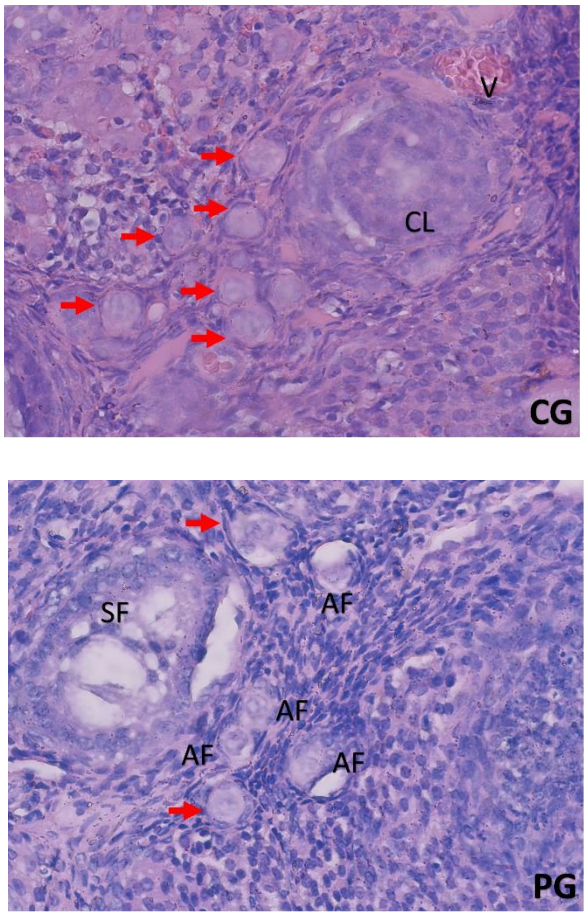

Figure 1. Histological examination of ovaries with 400x magnification. CG, ovaries of control group; PG, ovaries of periodontitis group; V, vascular; CL, corpus luteum; SF, secondary follicle; AF, atretic follicle; red arrow, primordial follicle

Figure 1 presents the findings from the histological examination of the ovaries in both research groups. The control group displayed a robust collection of primordial follicles, known as the primordial pool. In contrast, the periodontitis group also exhibited a primordial pool, but it contained only a limited number of primordial follicles, with many of them undergoing atresia.

The results indicated that the number of primordial follicles in the periodontitis group was lower than in the control group. The presence of *P. gingivalis* likely induced inflammation and infection in the ovaries. This condition might lead to damage, necrosis, and premature death of primordial follicles, resulting in a reduced number of these follicles in the ovaries. Infection of gram-negative bacteria and their endotoxins triggers inflammation in the ovaries, which causes necrosis in the cells that comprise the ovarian follicles,

subsequently decreasing the overall number of ovarian follicles. Fuller et al. demonstrated that lipopolysaccharides (LPS) from *Salmonella enterica* induced the premature activation of primordial follicles in the ovaries of mice, leading to a decrease in the number of primordial follicles that were unable to develop and mature. Moreover, Bromfield and Sheldon indicated that LPS of *E. coli* disrupted the development of primordial follicles by promoting the development of atretic follicles from primordial follicles. This occurrence further reduced the number of primordial follicles [14],[19]-[21]. Both *Salmonella enterica* and *E. coli* are similar to *P. gingivalis*, a Gram-negative bacterium with virulence factors akin to those of *P. gingivalis*, particularly LPS.

P. gingivalis possesses various virulence factors, including fimbriae, LPS, and gingipain. These factors damage periodontal tissues as well as tissues in other organs by disrupting junction proteins within the tissue [17],[22]. The destruction of junction proteins, particularly in the endothelium and epithelium, compromises tissue structure and facilitates the penetration and translocation of bacteria and their products to other tissues, including the ovaries [9],[13],[17].

The decrease in primordial follicles observed in this study due to *P. gingivalis* infection might be linked to the death of pre-granulosa cells. While the death of pre-granulosa and granulosa cells is physiology process of folliculogenesis, an excessive and premature loss of these cells poses a significant risk for disorders in this process [23]-[24]. The premature ovarian cell death was triggered by hormonal imbalances and inflammation, including that caused by *P. gingivalis* infection. This inflammation led to the formation of excessive atretic follicles, particularly in primordial follicles [24]-[26].

Furthermore, the reduction of primordial follicles resulting from *P. gingivalis* infection was also associated with the defense mechanisms of these follicles. Primordial follicles consist of only a single layer of flat pre-granulosa cells, making their simple structure particularly vulnerable to damage from *P. gingivalis* junction proteins and its virulence factors. This vulnerability triggers the formation of atretic follicles [14], [27],[28].

Abnormal death of primordial follicles due to *P. gingivalis* infection occurs when the bacteria and its virulence factors bind to the surface of granulosa cells' phosphoproteins (PPPs). This binding activates inflammatory signals from both immune competent and incompetent cells [29]. Consequently, inflammation stimulates the production of pro-inflammatory cytokines, including Interleukin-6 (IL-6), Interleukin-8 (IL-8), and Tumor Necrosis Factor Alpha (TNF-α) [30],[31]. The presence of these bacteria, their virulence factors, and the cytokines likely prompts premature follicle progression to the next stage of folliculogenesis, resulting in primordial follicle apoptosis or atresia due to the accumulation of pro-inflammatory cytokines in the ovaries [32],[33]. This leads to a reduction in the reserve of primordial follicles in the ovaries, thereby increasing the risk of infertility. The study suggests that *P. gingivalis* infection, as a major periodontal pathogen, can trigger disorders related to female reproductive health. However, it is important to note that this study did not explore other factors that may enhance the impact of *P. gingivalis* infection on ovarian health.

Conclusion

In summary, *P. gingivalis* infection led to a decrease in the number of primordial follicles in the ovaries of a female periodontitis model. However, further researches are needed to explore other factors related to the premature death of pregranulosa cells in primordial follicles and the increased atresia of follicles resulting from the early damage to primordial follicles.

Acknowledgements

The authors would like to express their gratitude to Universitas Jember for funding this study and to the Faculty of Medicine at Airlangga University for providing the necessary research facilities.

References

[1] A. W. S. Dharmayanti, and B. Kusumawardani, “Deoxypyridinoline and Mineral Levels in Gingival Crevicular Fluid as Disorder Indicators of Menopausal Women with Periodontal Disease,” *Dent. J. (Majalah Kedokt. Gigi)*, vol. 50, no. 32, 2017, pp. 131–137, doi: 10.20473/j.djmkg.v50.i3.p131-137.

[2] I. K. E. Wijaksana, “Periodontal Chart dan Periodontal Risk Assessment sebagai Bahan Evaluasi dan Edukasi Pasien dengan Penyakit Periodontal,” *J. Kesehat. Gigi*, vol. 6, no. 1,2019, p. 19, doi: 10.31983/jkg.v6i1.4032.

[3] G. G. Nascimento, S. Alves-Costa, and M.

Romandini, “Burden of Severe Periodontitis and Edentulism in 2021, with projections up to 2050: The Global Burden of Disease 2021 study,” *J. Periodontal Res.*, vol. 59, no. August, 2024, pp. 823–867, doi: 10.1111/jre.13337.

[4] M. X. Chen, Y. J. Zhong, Q. Q. Dong, H. M. Wong, and Y. F. Wen, “Global, Regional, and National Burden 0f Severe Periodontitis, 1990–2019: An analysis of the Global Burden of Disease Study 2019,” *J. Clin. Periodontol.*, vol. 48, no. 9, 2021, pp. 1165–1188, doi: 10.1111/jcpe.13506.

[5] E. Mardiati, A. Subekti, E. N. Donasari, and R. Oktaviani, “Perbedaan Ph Saliva terhadap Pemakai Kontrasepsi Hormonal Pil dan Suntik pada Wanita Usia 20-40 Tahun di Kelurahan Gedawang Kota Semarang,” *J. Kesehat. Gigi*, vol. 7, no. 1, 2020, pp. 13–20, doi: 10.31983/jkg.v7i1.5519.

[6] E. A. Ekoningtyas, I. H. Y. Siregar, and S. J. Sukendro, “the Effect of Giving Education about Rheological Saliva To Potential Quality of Mouth Saliva in Menopause Women,” *J. Kesehat. Gigi*, vol. 5, no. 2, 2018, p. 52, doi: 10.31983/jkg.v5i2.3862.

[7] T. R. Septiwidyati and E. W. Bachtiar, “The role of *Porphyromonas gingivalis* Proteinases in Periodontitis,” *dentika Dent. J.*, vol. 23, no. 1, 2020, pp. 6–12.

[8] Y. Martayuda, C. Kurnia, and V. K. Sugiaman, “Antibacterial Activity of Andaliman Ethanol Extract (*Zantoxylum acanthopodium Dc*.) Against *Porphyromonas gingivalis* Bacteria,” *J. Kesehat. Gigi*, vol. 10, no. 2, 2023, pp. 108–115, doi: 10.31983/jkg.v10i2.10866.

[9] C. A. de Jongh, T. J. de Vries, F. J. Bikker, S. Gibbs, and B. P. Krom, “Mechanisms of *Porphyromonas gingivalis* to Translocate over The Oral Mucosa and Other Tissue Barriers,” *J. Oral Microbiol.*, vol. 15, no. 1, 2023, doi: 10.1080/20002297.2023.2205291.

[10] A. W. S. Dharmayanti, S. Puspita, D. W. A. Fatmawati, and T. Ermawati, “Systematic Review: *Porphyromonas gingivalis* Gingipain Plays a Vital Role in Organ Dysfunction in Systemic Diseases,” *Proceeding Improv. Qual. Dent.*, vol. 1, no. 1, 2024, pp. 6–21, doi: 10.18196/imunity.v1i1.2.

[11] A. Nirola, P. Batra, and J. Kaur, “Ascendancy of Sex Hormones on Periodontium During Reproductive Life Cycle of Women,” *J. Int. Clin. Dent. Res. Organ.*, vol. 10, no. 1, 2018, p. 3, doi: 10.4103/jicdro.jicdro_29_17.

[12] J. Kotz, N. Chegini, K. T. Shiverick, and R. J. Lamont, “Localization of *P. gingivalis* in Preterm Delivery Placenta,” *J. Dent. Res.*, vol. 88, no. 6, 2009, pp. 575–578, doi: 10.1177/0022034509338032.

[13] L. A. Gómez *et al.*, “*Porphyromonas gingivalis* Placental Atopobiosis and Inflammatory Responses in Women With Adverse Pregnancy Outcomes,” *Front. Microbiol.*, vol. 11, no. December, 2020, doi: 10.3389/fmicb.2020.591626.

[14] J. J. Bromfield and I. M. Sheldon, “Lipopolysaccharide Reduces the Primordial Follicle Pool in the Bovine Ovarian Cortex Ex Vivo and in the Murine Ovary In Vivo1,” *Biol. Reprod.*, vol. 88, no. 4, 2013,pp. 1–9, doi: 10.1095/biolreprod.112.106914.

[15] N. Yamamoto *et al.*, “Lipopolysaccharide (LPS) Suppresses Follicle Development Marker Expression and Enhances Cytokine Expressions, Which Results in Fail to Granulosa Cell Proliferation in Developing Follicle in Cows,” *Reprod. Biol.*, vol. 23, no. 1, 2023, p. 100710, doi: 10.1016/j.repbio.2022.100710.

[16] Rongkun Liu, T. Desta, M. Raptis, R. P. Darveau, and D. T. Graves, “P*orphyromonas gingivalis* and *E. coli* Lipopolysaccharide Exhibit Different Systemic but Similar Local Induction of Inflammatory Markers,” *J Periodontol.*, vol. 79, no. 7, 2008, pp. 1241–1247, doi: 10.1902/jop.2008.070575.

[17] A. W. S. Dharmayanti, Z. Meilawaty, T. Ermawati, A. M. Putradjaka, and Z. Hamzah, “Effect of Ovarian Dysfunction Induced Ovariectomy and *Porphyromonas gingivalis* Induction To Risk of Metabolic Syndrome: In Vivo Study,” *Brazilian Dent. Sci.*, vol. 27, no. 1, 2024, pp. 1–12, doi: 10.4322/bds.2024.e3880.

[18] A. W. S. Dharmayanti *et al.*, “Superoxide Dismutase Expression in the Ovaries of Periodontitis Animal Models Induced by *Porphyromonas gingivalis* and Treated with Cassava Leaves Extract,” *Ann. Dent.*, vol. 28, 2021, pp. 40–46, doi: 10.22452/adum.vol28no7.

[19] E. A. Fuller *et al.*, “Neonatal Immune Activation Depletes The Ovarian Follicle Reserve and Alters Ovarian Acute Inflammatory Mediators in Neonatal Rats,” *Biol. Reprod.*, vol. 97, no. 5, 2017, pp. 719–730, doi: 10.1093/biolre/iox123.

[20] L. Sominsky, A. P. Sobinoff, M. S.Jobling, V. Pye, E. A. McLaughlin, and D. M. Hodgson, "Immune Regulation of Ovarian Development : Programming by Neonatal Immune Challenge," *Front. Neurosci.*, vol. 7, no. June, 2013, pp. 1–20, doi: 10.3389/fnins.2013.00100.

[21] A. S. Maffi *et al.*, "Lipopolysaccharide effects On Activation aand Apoptosis Of Primordial Ovarian Follicles in Heifers," *Arq. Bras. Med. Vet. e Zootec.*, vol. 75, no. 3, 2023, pp. 381–390, doi: 10.1590/1678-4162-12848.

[22] H. Takeuchi, E. Nakamura, S. Yamaga, and A. Amano, "*Porphyromonas gingivalis* Infection Induces Lipopolysaccharide and Peptidoglycan Penetration Through Gingival Epithelium," *Front. oral Heal.*, vol. 3, no. February, 2022, pp. 1–6, doi: 10.3389/froh.2022.845002.

[23] M. K. Y. Siu and C. Y. Cheng, "The Blood-Follicle Barrier (BFB) in Disease and in Ovarian Function," in *Biology and Regulation of Blood-Tissue Barriers*, 2013, pp. 186–192.

[24] M. K. Y. Siu and C. Y. Cheng, "the Blood-Follicle Barrier (BFB) in Disease and in Ovarian Function Introduction: the Concept of the Blood-Follicle Barrier," *Adv Exp Med Biol*, vol. 763, 2012, pp. 1–8.

[25] C. Lliberos, S. H. Liew, P. Zareie, N. L. La Gruta, A. Mansell, and K. Hutt, "Evaluation of Inflammation and Follicle Depletion During Ovarian Ageing in Mice," *Sci. Rep.*, vol. 11, no. 278, 2021, pp. 1–15, doi: 10.1038/s41598-020-79488-4.

[26] Z. Zhang, L. Huang, and L. Brayboy, "Macrophages: An Indispensable Piece of Ovarian Health," *Biol. Reprod.*, vol. 104, no. 3, 2021, pp. 527–538, doi: 10.1093/biolre/ioaa219.

[27] Z. Zhu *et al.*, "Pregranulosa Cell–Derived FGF23 Protects Oocytes from Premature Apoptosis During Primordial Follicle Formation By Inhibiting p38 MAPK in Mice," *J. Biol. Chem.*, vol. 299, no. 6, 2023, p. 104776, doi: 10.1016/j.jbc.2023.104776.

[28] Y. L. Chu, Y. R. Xu, W. X. Yang, and Y. Sun, "The Role of FSH and TGF-β Superfamily in Follicle Atresia," *Aging (Albany. NY).*, vol. 10, no. 3, 2018, pp. 305–321.

[29] I. Olsen, J. D. Lambris, and G. Hajishengallis, "*Porphyromonas gingivalis* disturbs Host–Commensal Homeostasis by Changing Complement Function," *J. Oral Microbiol.*, vol. 9, no. 1, 2017, doi: 10.1080/20002297.2017.1340085.

[30] A. Gram and M. P. Kowalewski, "Molecular Mechanisms of Lipopolysaccharide (LPS) Induced Inflammation in an Immortalized Ovine Luteal Endothelial Cell Line (OLENDO)," *Vet. Sci.*, vol. 9, no. 3, 2022, doi: 10.3390/vetsci9030099.

[31] V. Charoensaensuk, Y.-C. Chen, Y.-H. Lin, K.-L. Ou, L.-Y. Yang, and D.-Y. Lu, "*Porphyromonas gingivalis* Induces Proinflammatory Cytokine Expression Leading to Apoptotic Death through the Oxidative Stress/NF-κB Pathway in Brain Endothelial Cells," *cells*, vol. 10, no. 3033, 2021, pp. 1–21, doi: https://doi.org/10.3390/cells10113033.

[32] C. K. Yeung *et al.*, "BRE Modulates Granulosa Cell Death to Affect Ovarian Follicle Development and Atresia In The Mouse," *Cell Death Dis.*, vol. 8, no. 3, pp. 1–11, 2017, doi: 10.1038/cddis.2017.91.

[33] K. L. Bidne, M. J. Dickson, J. W. Ross, L. H. Baumgard, and A. F. Keating, "Disruption of Female Reproductive Function by Endotoxins," *Reproduction*, vol. 155, no. 4, 2020, pp. R169–R181, doi: 10.1530/REP-17-0406.