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Original Article

The effect of laserpuncture shooting on the histomorphometry of the bursa of Fabricius in pullets (*Gallus gallus*) that lay eggs late

Ayu Kamila Tiarani[1], Widya Paramita Lokapirnasari[2*], Soeharsono Soeharsono[3]

[1]Profession Program of Veterinary Medicine, Faculty of Veterinary Medicine, Universitas Airlangga, Jl. Dr. Ir. H. Soekarno, Kampus C Mulyorejo, Surabaya 60115, East Java, Indonesia
[2]Division of Animal Husbandry, Faculty of Veterinary Medicine, Universitas Airlangga, Jl. Dr. Ir. H. Soekarno, Kampus C Mulyorejo, Surabaya 60115, East Java, Indonesia
[3]Division of Veterinary Anatomy, Faculty of Veterinary Medicine, Universitas Airlangga, Jl. Dr. Ir. H. Soekarno, Kampus C Mulyorejo, Surabaya 60115, East Java, Indonesia

ABSTRACT

The continuing increase in egg consumption has led to the need to ensure that the laying hen industry sector is able to keep up with the need for eggs, one way is by increasing the productivity of laying hens and ensuring that laying hens can lay eggs on time. This study aims to determine the number and diameter of the bursa of Fabricius lymphoid follicles in late-laying pullets laserpuncture. This research is a 2x4 factorial complete randomized design experimental study. Chickens were divided into two groups, First Grade and Second Grade. Each Grade group was further divided into four groups based on laserpuncture points, namely control, Ova, weigen, and Ova-weigen. The variables observed were the diameter and number of lymphoid follicles of the bursa of Fabricius microscopically. The observation data were analyzed statistically using analysis of variance (ANOVA) followed by the Duncan test. The results of the analysis showed that 0.5 Joule laserpuncture had a significant effect on the diameter and number of the bursa of Fabricius lymphoid follicles ($p<0.05$). Laserpuncture shooting 0.5 Joules at the Wei Gen point shows the smallest diameter of the lymphoid follicles. While Ova point showed the smallest number of bursa Fabricius lymphoid follicles.

Keywords: Bursa Fabricius, laserpuncture, follicle diameter, follicle number, pullet.

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widya-pl@fkh.unair.ac.id
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Introduction

Eggs are a nutritious, inexpensive, multi-functional, and long-lasting source of animal protein (Myers and Ruxton, 2023). The advantages of eggs over other animal protein sources lie in their high nutritional content, delicious taste, easy availability, and ability to be processed into various foods at a relatively affordable price. Chicken eggs contain several nutrients, including protein (12.8%), fat (11.8%), vitamin A (327.0 IU), and minerals (256.0 mg) (Wallace *et al.*, 2023).

A common problem in laying hen farms is delayed egg production. A survey conducted in 30 pens owned by PT. Unggul Indah found that in 19-week-old pullets, egg production only reached 2-5%, even though at that age the productivity should have reached 10%. Delayed egg production can result in losses for farmers and an inability to meet the community's egg demand (Caputo *et al.*, 2023). Therefore, efforts are needed to ensure timely egg production for pullets. One effort that can be made to ensure timely egg production is the application of laser puncture.

Laserpuncture is a variation of acupuncture that utilizes low-intensity, nonthermal laser radiation to stimulate acupuncture points in livestock (Chon *et al.*, 2019). The laser can influence various processes at the cellular level, such as the release of growth factors, cell proliferation, and cellular activity, leading to faster healing and regeneration (Litscher, 2024). Several studies have shown that laserpuncture can improve the circulatory, endocrine, and various other systems in the body (Tam *et al.*, 2020).

The Wei Gen point (bursa Fabricius point) is an acupuncture point intended to enhance humoral immune response activity (Adikara, 2018). The effect of laserpuncture depends on the dose used, with stimulation being used to stimulate and sedation being used to calm (Yoon *et al*., 2022). Laserpuncture with a sedative dose at the Wei Gen point in pullets that are late in laying eggs will actually impact the involution of the bursa Fabricius. Involution of the bursa Fabricius is characterized by a decrease in the number and diameter of lymphoid follicles in the bursa Fabricius (Cheng *et al*., 2023).

Laserpuncture depends on the dosage used, with stimulation and sedation (Yoon *et al*., 2022). Laserpuncture with a sedation dose at the Wei Gen point in late-laying pullets can actually impact the involution of the bursa of Fabricius. This is characterized by a decrease in the number and diameter of lymphoid follicles in the bursa of Fabricius (Cheng *et al*., 2023).

The involution of the bursa of Fabricius is observed through histomorphometry of the diameter and number of lymphoid follicles of the bursa of Fabricius (Abdel-Fattah *et al.*, 2025). The bursa of Fabricius is expected to undergo involution, and the estrogen hormone in pullets is increased, ultimately accelerating the onset of egg-laying. This study aims to determine the number and diameter of the bursa of Fabricius lymphoid follicles in late-laying pullets with laser puncture. Laser puncture application at the Ova, Wei Gen, and Ova and Wei Gen points in this study is expected to induce involution of the bursa of Fabricius in late-laying pullets aged 17 to 21 weeks.

Materials and methods

Research design

This research was conducted experimentally through several stages over a four-month period, from April 19 to August 18, 2024, at the Faculty of Veterinary Medicine, Airlangga University, Surabaya. Pullets were raised, and laser puncture was performed in the Experimental Animal Cage. Pullet euthanasia and bursa of Fabricius organ removal were performed in the Anatomy Laboratory. Histological slides of the bursa of Fabricius were prepared and observed in the Pathology Laboratory. The research sample consisted of 24 17-week-old pullets, each group consisting of three chickens.

This research is an experimental research with a completely randomized design with a 2x4 factorial. The first factor is the prediction of the initial laying time of pullet chickens, which are divided into two Grades, namely First Grade and Second Grade, based on the morphometry of the comb, wattle, and body weight. Pullet chickens whose morphometry of the comb, wattle, and body weight has been measured are sorted, and the top 12 chickens are grouped as the First Grade group, while the remaining 12 chickens are grouped as the Second Grade. The second factor is the laserpuncture point, which is divided into four groups, namely Control, Ova, Wei Gen, and Ova and Wei Gen, so that the total group of this study is eight.

Preparation of pullet chickens

Sixteen-week-old pullets were kept in 120 x 35 x 57 cm battery cages equipped with feeders and waterers. They were fed 100 grams of Super Cargill layer concentrate with 17% protein once daily at 1:00 p.m., and water was provided ad libitum. The pullets were exposed to light from 7:00 a.m. to 4:00 p.m. for a total of nine hours. The experimental animals were acclimatized for one week before treatment to avoid stress and accustom them to their new environment.

Group division

The 16-week-old pullets were acclimatized for one week. The first treatment involved 17-week-old pullets, who had already undergone a one-week adaptation period, which began at 16 weeks. The pullets were divided into two groups based on predicted egg-laying age, based on comb, wattle, and body weight morphometry. These groups were then further subdivided into First Grade and Second Grade.

Then, chickens from each Grade group were randomly divided into four groups, namely

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G1A0 as First Grade Control, G1A1 as First Grade with 0.5 joule laserpuncture for 50 seconds at the Ova point, G1A2 as First Grade with 0.5 joule laserpuncture for 50 seconds at the Wei Gen point, G1A3 as First Grade group with 0.5 joule laserpuncture for 50 seconds at the Ova and Wei Gen points, G2A0 as Second Grade Control group, G2A1 as Second Grade group with 0.5 joule laserpuncture for 50 seconds at the Ova point, G2A2 as Second Grade group with 0.5 joule laserpuncture for 50 seconds at the Wei Gen point, and G2A3 as Second Grade group with 0.5 joule laserpuncture for 50 seconds at the Ova and Wei Gen points (Table 1).

Table 1. Experimental animal treatment group

Acupuncture Point (A)	Grade (G)	
	1st Grade (G1)	2nt Grade (G1)
Control (A0)	G1A0	G2A0
Ova (A1)	G1A1	G2A1
Wei Gen (A2)	G1A2	G2A2
Ova dan Wei Gen (A3)	G1A3	G2A3

Acupuncture point detection

Acupuncture points are identified using the Acu-Doctor Electro Acupuncture Pen (Accupointer) by attaching the device to the chicken's skin. The device will beep when the acupuncture point is found. The detected points are the Ova point, which is the reproductive point, and the Wei Gen point, which is a defense point in chickens that stimulates the bursa of Fabricius (Figure 1).

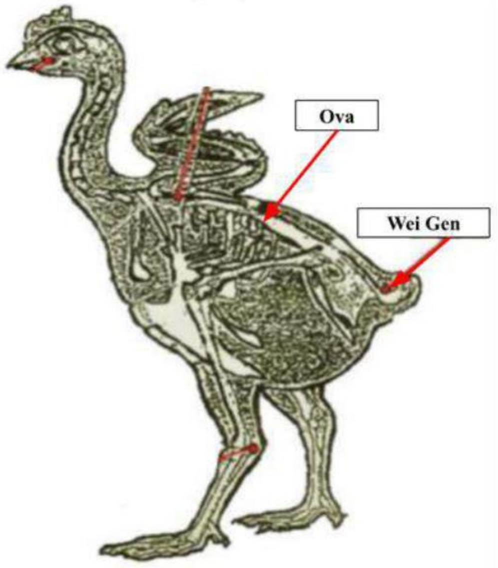

Figure 1. Location of acupuncture points on poultry

Treatment of pullet chickens

Laserpuncture was performed using a 20 mW semiconductor laserpuncture device with a dose of 0.5 Joules for 50 seconds at the Ova, Wei Gen, and Ova Wei Gen laserpuncture points, according to their respective groups. The laserpuncture dose administered at each point was 0.5 Joules, which is considered a sedative dose, with the aim of stimulating involution of the bursa of Fabricius. Treatment was performed once a week and repeated weekly for five weeks.

Bursa Fabricius stock sampling

After all treatments were completed, the pullets were euthanized by slaughter, which involved cutting the airways, foodways, and blood vessels, including the carotid artery and jugular vein, in the chicken's neck (Ahmed et al., 2018). The bursa of Fabricius was then surgically removed. The removed bursa of Fabricius was then placed in 10% Neutral Buffered Formalin.

Preparation of histological preparations

The preparation of histological preparations is carried out in several stages, namely fixation, dehydration, clearing, infiltration, deparaffinization, staining, and mounting (Isaac *et al*., 2023). The collected bursa of Fabricius samples are fixed by storing the organs in 10% Neutral Buffered Formalin for 14-48 hours. The next stage is tissue dehydration using alcohol with graded concentrations starting from 70%, 80%, 90%, and 95%, absolute alcohol I, II, and III. Next, the clearing process is carried out using xylene I, II, and III. The tissue in the tissue basket is then placed in liquid paraffin I, II, and III for the paraffin infiltration process. The paraffin infiltration process uses hot paraffin wax at a temperature of 56-58ºC (Al-Sabawy *et al*., 2021). After that, the organs are embedded in a cooled liquid paraffin mold.

The paraffin blocks containing the organs were then sliced using a microtome with a thickness of 3-4 μm. The sections were then placed on a glass slide and deparaffinized using xylene solution and rehydrated with graded alcohol concentrations of 100% (III, II, and I), 95%, 90%, 80%, and 70%, then immersed in water for 10 minutes. The next step was to stain the slides with Hematoxylin-Eosin. The stained slides were rehydrated with graded alcohol and then cleared using xylene. The final step was the mounting process, which involved covering the slides with a cover glass using adhesive/entelan to preserve the stained slides (Saify and Tiwari, 2020).

Histological observation

The finished histological preparations of the bursa of Fabricius were observed using a Nikon Eclipse E100 microscope fitted with an Optilab. The dependent variables observed microscopically were the number and diameter of lymphoid follicles in the bursa of Fabricius of pullets. Observations were made in five fields of view of each preparation at 40x magnification (Islam *et al*., 2019).

Measurement of the diameter of the bursa of Fabricius follicles was performed using Image Raster software. Follicle diameter was measured by adopting the method of Wahyuwardani *et al*. (2015), namely by averaging the results of several line measurements from the farthest and closest sides of five follicles obtained from five different fields of view in each preparation.

Data analysis

The research data are generally presented in tables as means and standard deviations. To determine differences between groups, a two-way analysis of variance (ANOVA) with interaction was performed using IBM SPSS Statistics 27. If there was an interaction between treatment groups, a Duncan test was performed. The best treatment group was the one with the smallest number and diameter of lymphoid follicles because the expected outcome of this study was the involution of the bursa of Fabricius, characterized by a reduction in follicle diameter and a reduction in the number of follicles within the bursa of Fabricius.

Result

Histological observation

The study was conducted on two Grade groups, namely First Grade and Second Grade based on the morphometry of the comb, wattle, and body weight. G1A0 as the First Grade Control group, G1A1 as the First Grade group with the administration of 0.5 joule laserpuncture for 50 seconds at the Ova point, G1A2 as the First Grade group with the administration of 0.5 joule laserpuncture for 50 seconds at the Wei Gen point, G1A3 as the First Grade group with the administration of 0.5 joule laserpuncture for 50 seconds at the Ova and Wei Gen points (Figure 2). G2A0 as the Second Grade Control group, G2A1 as the Second Grade group with the administration of 0.5 joule laserpuncture for 50 seconds at the Ova point, G2A2 as the Second Grade group with the administration of 0.5 joule laserpuncture for 50 seconds at the Wei Gen point, and G2A3 as the Second Grade group with the administration of 0.5 joule laserpuncture for 50 seconds at the Ova and Wei Gen points (Figure 3).

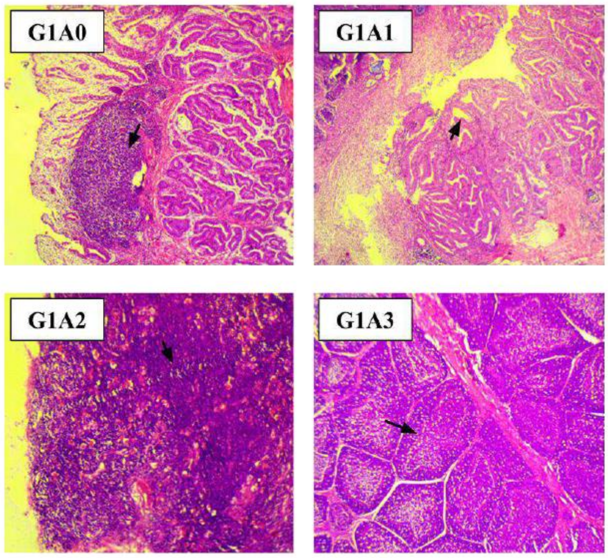

Figure 2. First Grade bursa of Fabricius contains lymphoid follicles (arrow). Hematoxylin-Eosin (HE) staining at 40x magnification

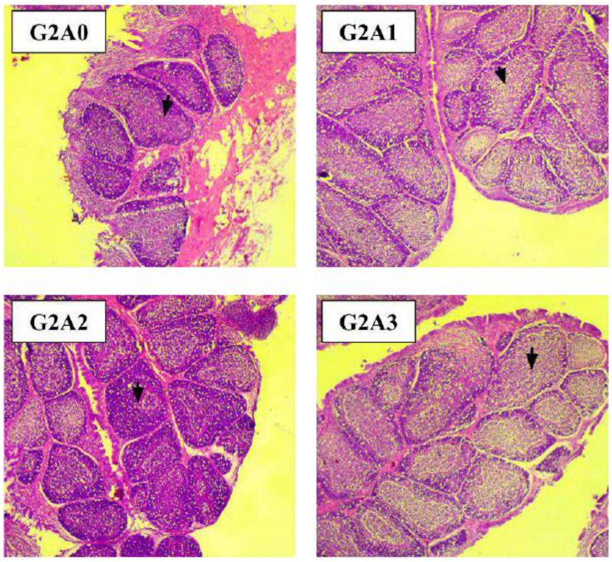

Figure 3. Bursa of Fabricius of the Second Grade group contains lymphoid follicles (arrows). Hematoxylin-Eosin (HE) staining at 40x magnification

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Diameter of lymphoid follicles of the bursa Fabricius

The effect of laserpuncture on the diameter of the lymphoid follicles of the bursa of Fabricius can be observed in Table 2. The results of the Two-Way ANOVA test of the diameter of the lymphoid follicles of the bursa of Fabricius showed p<0.05, so that there was a significant difference between the control, Ova, Wei Gen, and Ova-Wei Gen treatment groups, and there was an interaction between Grade and the laserpuncture point. The analysis was continued with a Duncan test at a level of 5% to determine the groups that were significantly different (Figure 4).

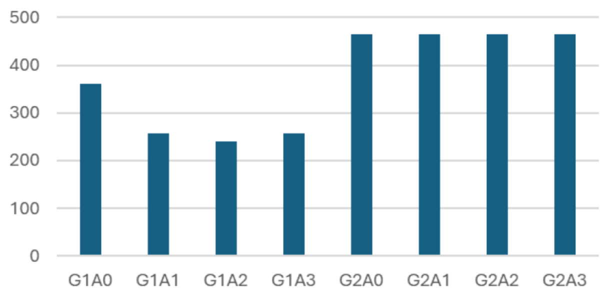

Figure 4. The average diameter of the lymphoid follicles of the bursa of Fabricius from each treatment

In the control group, there was a significant difference between the First Grade control group and the Second Grade control group (p<0.05). The First Grade control group showed a significant difference with the Ova, Wei Gen, and Ova-Wei Gen First Grade groups (p<0.05), but was not significantly different from the Ova, Wei Gen, and Ova-Wei Gen groups of Second Grade (p>0.05).

Table 2. Mean and standard deviation of the measurement results of the diameter of the lymphoid follicles of the bursa of Fabricius

Treatment	Diameter of Lymphoid Follicle of Bursa of Fabricius (µm)
G1A0	$359.94^{b} \pm 47.99$
G1A1	$257.74^{a} \pm 44.88$
G1A2	$239.73^{a} \pm 33.35$
G1A3	$256.58^{a} \pm 49.13$
G2A0	$465.45^{c} \pm 22.18$
G2A1	$332.76^{b} \pm 50.38$
G2A2	$364.06^{b} \pm 12.15$
G2A3	$358.45^{b} \pm 6.51$

Note: Different superscripts in the same column indicate significant differences (p<0.05).

The Second Grade Control group showed significant differences with the Ova, Wei Gen, and Ova-Wei Gen groups from both the First Grade and Second Grade groups (p<0.05). The Ova, Wei Gen, and Ova-Wei Gen groups from First Grade did not show significant differences between each other because they were in the same subset (p>0.05). Likewise, the Ova, Wei Gen, and Ova-Wei Gen groups from Second Grade were all in the same subset (p>0.05). However, the Ova, Wei Gen, and Ova-Wei Gen groups from First Grade were significantly different from the Ova, Wei Gen, and Ova-Wei Gen groups from Second Grade (p<0.05).

Number of lymphoid follicles of the bursa Fabricius

The results of the two-way ANOVA test on the number of lymphoid follicles of the bursa of Fabricius showed p<0.05, so that there was a significant difference between the control, Ova, Wei Gen, and Ova-Wei Gen treatment groups. The analysis was continued with a Duncan test at a level of 5% to determine which groups were significantly different (Figure 5).

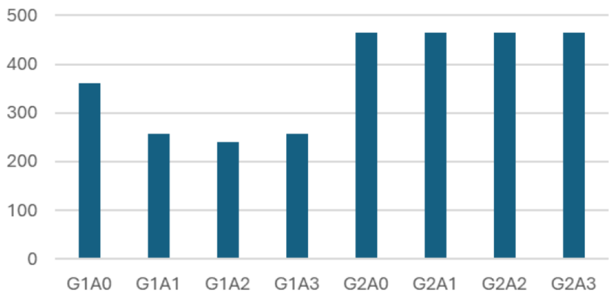

Figure 4. The average diameter of the lymphoid follicles of the bursa of Fabricius from each treatment

The First Grade Control group showed a significant difference with the First Grade Ova group (p<0.05), but did not show a significant difference with the Wei Gen and Ova-Wei Gen First Grade groups, and the Control, Ova, Weigen, and Ova-Wei Gen Second Grade groups (p>0.05). The First Grade Ova group showed a significant difference with the Control and Wei Gen First Grade, Control, Ova, Wei Gen, and Ova-Wei Gen Second Grade groups (p<0.05), but did not show a significant difference with the Ova-Wei Gen First Grade group (p>0.05). The First Grade Wei Gen group was significantly different from the Ova First Grade and Wei Gen Second Grade groups ($p<0.05$), but was not significantly different from the Control and Ova-Wei Gen First Grade, Control, Ova, and Ova-Wei Gen Second Grade groups ($p>0.05$). The Ova Wei Gen First Grade group was significantly different from the Control, Wei Gen, and Ova-Weigen Second Grade groups ($p<0.05$), but not significantly different from the Control, Ova, and Wei Gen First Grade groups ($p>0.05$).

The Second Grade Control group was significantly different from the Ova and Ova-Wei Gen First Grade groups ($p<0.05$), but did not show significant differences with the Control and Wei Gen First Grade, Ova, Wei Gen, and Ova-Wei Gen Second Grade groups ($p>0.05$). The Second Grade Ova group was significantly different from the Ova First Grade and Wei Gen Second Grade groups ($p<0.05$), but was not significantly different from the Control, Wei Gen, and Ova-Wei Gen First Grade, Control and Ova-Wei Gen Second Grade groups ($p>0.05$). The Wei Gen Second Grade group was significantly different from the Ova, Wei Gen, and Ova-Wei Gen First Grade groups, and the Ova Second Grade group ($p<0.05$), but was not significantly different from the Control First Grade, Control and Ova-Wei Gen Second Grade groups ($p>0.05$). The Ova-Wei Gen Second Grade group was significantly different from the Ova and Ova-Wei Gen First Grade groups ($p<0.05$), but was not significantly different from the Control, Wei Gen First Grade, Control, Ova, Wei Gen Second Grade groups ($p>0.05$).

Discussion

The effect of laserpuncture on the diameter of lymphoid follicles in the bursa of Fabricius

The data in Table 2 shows that the average size of the lymphoid follicle diameter of the bursa of Fabricius shows that the group given a laser shot at a dose of 0.5 Joules at the Wei Gen First Grade point has the smallest average lymphoid follicle diameter, namely 239.73 ± 33.351 μm. The small follicle diameter value indicates that in the Wei Gen First Grade group, the bursa of Fabricius experienced the greatest decrease in the number of follicles compared to the bursa of Fabricius of chickens in other treatment groups.

The Wei Gen point is an acupuncture point used to stimulate the immune system in poultry. It is located on the dorsal side of the sacrococcygeal joint and is close to the bursa of Fabricius. Adikara (2018) stated that laserpuncture stimulation at the Wei Gen point can increase antibodies by delivering energy from the laser and triggering the organs to respond to that energy, resulting in physiological and biochemical changes such as increased organ function and capacity. Laserpuncture at the Wei Gen point can also restore the normal physiological function of the bursa of Fabricius. Bursa Fabricius merupakan organ limfoid istimewa yang hanya dapat ditemui pada unggas. Produksi dan pendewasaan limfosit B terjadi di dalam bursa Fabricius (Tizard, 2004). The mucosa of the bursa of Fabricius consists of folds filled with lymphoid follicles. Lymphoid follicles can be divided into two parts: the cortex and the medulla. The cortex of the bursa of Fabricius follicles is composed of cells derived from the mucosal epithelial cells of the bursa of Fabricius, while the medulla is composed of lymphoid cells (Udoumoh *et al.*, 2022). Interfollicular epithelium (IFE) and Follicle-associated Epithelium (FAE) are two epithelial components associated with the structure of lymphoid follicles in the bursa of Fabricius. IFE and FAE play a role in the formation and maturation of B lymphocytes. Katayama *et al*. (2012) found intense estrogen receptors in the IFE and FAE. The number of estrogen receptors in the IFE and FAE is proportional to the size of the bursa of Fabricius follicles. The diameter of the bursa of Fabricius follicles that have not undergone involution is larger than the diameter of the bursa of Fabricius follicles that have undergone involution, so the number of estrogen receptors in the bursa of Fabricius that has not undergone involution is greater. The large number of estrogen receptors in the bursa of Fabricius causes some of the estrogen that should stimulate reproductive organ development to actually affect the involution process in the bursa of Fabricius. As a result, reproductive organ development, such as ovarian follicle maturation and ovulation, is disrupted and suboptimal, ultimately delaying sexual maturity in hens.

All treatment groups, both First Grade and Second Grade, showed involution of the bursa of

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Fabricius. Involution is the return of the bursa of Fabricius to its original size after the organ has reached its maximum growth (Fang and Peng, 2014). An involuted bursa of Fabricius undergoes atrophy, leading to a reduction in the size of the bursa's lymphoid follicles. The final stage of involution is the regression stage, leading to a decrease in the number of lymphoid follicles in the bursa of Fabricius (Akbar *et al.*, 2012). Involuted bursa of Fabricius can be seen from a decrease in the number and diameter of lymphoid follicles, the accumulation of connective tissue, mucoid cysts, and a decrease in the thickness of the cortex and medulla of the bursa of Fabricius follicles (Cheng *et al.*, 2023).

The effect of laserpuncture on the number of lymphoid follicles in the bursa of Fabricius

The data presented in Table 3 shows that the average number of lymphoid follicles in the bursa of Fabricius from the Second Grade control group was 113.67 ± 31.50. This value is the highest compared to the average number of follicles in all other treatment groups. The smallest average number of lymphoid follicles in the bursa of Fabricius was seen in the First Grade Ova group, with a total of 13.00 ± 7.94. The bursa of Fabricius of chickens from the Ova group experienced the greatest decrease in follicle count compared to the other groups.

Table 3. Number of lymphoid follicles in bursa of fabricius at two grades and three acupuncture points

Treatment	Diameter of Lymphoid Follicle of Bursa of Fabricius (μm)
G1A0	$89.33^{bcd} \pm 4.51$
G1A1	$13.00^{a} \pm 7.94$
G1A2	$79.67^{bc} \pm 6.66$
G1A3	$46.67^{ab} \pm 3.79$
G2A0	$113.67^{cd} \pm 31.50$
G2A1	$75.67^{bc} \pm 8.33$
G2A2	$132.00^{d} \pm 58.62$
G2A3	$104.00^{cd} \pm 20.42$

Note: Different superscripts in the same column indicate significant differences ($p<0.05$).

The bursa of Fabricius follicles are the site of B cell production in birds, specifically in the cortex and medulla of the lymphoid follicles of the bursa of Fabricius (Cheng *et al.*, 2023). During embryonic development, the cellularity of the bursa tissue increases to 1 x 10^7 cells. B cells of the bursa of Fabricius during embryonic development are arranged within lymphoid follicles that still lack a clear structure. The structure of the lymphoid follicles of the bursa of Fabricius will change and develop into mature follicles as hatching approaches (Ratcliffe, 2006). Research by Olah and Glick (1978) found that in four-week-old chickens, each fold of the bursa of Fabricius mucosa is filled with approximately 800 lymphoid follicles. The bursa of Fabricius of chickens has an average of 10-15 folds, so the average number of follicles in the bursa of Fabricius in four-week-old chickens is approximately 8,000-12,000 follicles per bursa (Olah and Glick, 1978). The number of follicles increases and peaks when the chicken reaches sexual maturity, which is typically four months after hatching (Mfoundou *et al.*, 2021). The bursa of Fabricius then undergoes gradual involution and regression, accompanied by a decrease in the number and diameter of lymphoid follicles.

Wu *et al.* (2013) stated that failure of the bursa of Fabricius to involute can lead to decreased estrogen production, which can disrupt the chicken's reproductive process and inhibit egg-laying. The mucosa of the bursa of Fabricius contains estrogen receptors located in the lymphoid follicles, stroma, and epithelial areas (Katayama *et al.*, 2012). Estrogen receptors within the bursa are predominantly found in the follicle-associated epithelium (FAE) and interfollicular epithelium (IFE). Estrogen receptors in the bursa of Fabricius cause estradiol production by the ovaries to be directed not only to the reproductive organs but also to the bursa of Fabricius (Shin *et al.*, 2012). This results in some of the estrogen, which should stimulate reproductive organ development, instead affecting the involution of the Bursa of Fabricius. As a result, reproductive organ development processes, such as ovarian follicle maturation and ovulation, are disrupted and do not develop optimally, which can delay sexual maturity in hens.

The Ova point stimulates the ovaries to produce estrogen. Estrogen is a sex hormone produced in the ovaries and plays a crucial role in controlling female reproduction. One form of estrogen, estradiol-17β, is the primary estrogen

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involved in sexual maturity in hens (Hanlon *et al.*, 2022). Laser puncture stimulation of the Ova point increases ovarian estrogen production through peripheral nerve stimulation at the Ova point, which stimulates the hypothalamus to produce Gonadotropin-Releasing Hormone (GnRH) (Renata *et al.*, 2019). GnRH plays a role in estrogen production through the Hypothalamic-Pituitary-Gonadal (HPG) axis, where it stimulates the production of Follicle-Stimulating Hormone (FSH) and Luteinizing Hormone (LH) (Kaprara and Huhtaniemi, 2018). FSH will stimulate the development and growth of ovarian follicles and subsequently the synthesis of the hormone estrogen (Renata *et al.*, 2019).

The effect of grade on the diameter and number of lymphoid follicles in the bursa of Fabricius

Second Grade pullets have smaller comb and wattle morphometric measurements than First Grade pullets. Comb and wattle are important secondary sexual characteristics for measuring sexual maturity and are closely related to reproductive performance (Cai, *et al.*, 2024). Comb and wattle can function as markers indicating hormone levels in the chicken's body (Mukhtar and Khan, 2012). Increased estrogen levels in hens stimulate enlargement and color changes in the comb and wattle, indicating sexual maturity and reproductive readiness (Joseph *et al.*, 2003). Low morphometric and body weight values indicate low estrogen levels in the chicken's body because estrogen levels affect comb and wattle growth and the timing of first egg laying (Prastiya *et al.*, 2022).

The hormone estrogen, in addition to its role in reproduction, also plays a role in fat metabolism (Ren *et al.*, 2021). Fat accumulation in hens increases with increasing estrogen levels (Listyasari *et al.*, 2022). The liver is the primary organ for lipid metabolism in chickens. Estrogen, particularly estradiol-17β, has been shown to induce the expression of new genes related to lipid metabolism in the chicken liver, mediated by the estrogen receptor alpha (ERα) (Gitkind *et al.*, 2014). Low estrogen levels result in suboptimal fat metabolism, resulting in lower body weight in second-grade chickens (Zhang *et al.*, 2014).

In this study, applying laser puncture to the Ova point for five weeks triggered increased estrogen production in pullets. The resulting estrogen then stimulated growth of the reproductive tract and increased levels of calcium, protein, fat, and other components needed for egg formation (Hanlon *et al.*, 2022).

Conclusion

Based on the research results, it can be concluded that Grade can affect the decrease in the diameter and number of lymphoid follicles of the bursa of Fabricius in late-laying pullets (*Gallus gallus*). Laserpuncture at the Ova, Wei Gen, and Ova and Wei Gen points can reduce the diameter and number of lymphoid follicles of the bursa of Fabricius in late-laying pullets (*Gallus gallus*). There is an interaction between Grade of pullets and laserpuncture at the Ova, Wei Gen, and Ova and Wei Gen points in reducing the diameter and number of lymphoid follicles of the bursa of Fabricius in late-laying pullets (*Gallus gallus*).

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