Conference Paper

Effect of Laser Acupuncture Shoot on Ova Point of Male Mojosari Duck (*Anas plathyrhynchos*) on The Number of Sertoli and Leydig Cells

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Abstract

Demand for duck eggs and meat recently increased along with the increasing interest of the community to consume duck eggs and meat. Due to that reason researcher try to find the best way to enhance the reproductive ability of livestock., which is in this research by laser puncture shot. Eighteen ducks were allocated into three groups, the control group, the first treatment group with laserpuncture shoot dose of 0.2 Joule (J), and the second treatment group with laser puncture shoot dose of 0.4 J. Data were analyzed with ANOVA and further tested by Duncan multiple range test. Research result showed that laser puncture shoot with a dose of 0.2 J and 0.4 J decreased the number of Sertoli and Leydig cells.

Keywords: Mojosari duck; laser acupuncture; Sertoli cells; Leydig cells.

1. Introduction

Food is a critical issue currently faced by all countries in the world. In 2013 commotion occurred in the Indonesian market regarding the price of meat, especially beef. The issue arises because the demand for beef continued increasing [1]. Besides focusing on the procurement of beef, poultry meat turned out to give a significant role in the availability of national meat. Development of poultry farm is also a priority because

public demand for poultry increased considering the growth and production of poultry is faster than ruminants or non poultry [2].

Demand for duck eggs and meat recently increased along with the increasing interest of the community to consume eggs and duck meat. Public interest in consuming duck meat is almost equivalent to those of in chicken meat. This increasing demand needs to be balanced with the provision of the quality of seedlings duck in quantity and sustainability. The duck breeding stock supply will indirectly support the development of duck farms and encourage the creation of technology that affects ducks growth.

The use of alternative technologies as the intensification duck breeding in order to obtain the expected results have been necessary [3]. Laser acupuncture shoot on GV-4 point, in both male and female garut sheep, gave the positive effect that was an improvement of reproductive organ function and reproductive acupuncture point in both male and female sheep were in the same place) [4]. Laser utilization in livestock aimed at improving the biological balance and health of livestock, besides the important thing is to improve the productivity of livestock and can also enhance the reproductive ability of livestock. To achieve the above objectives, the laser is fired at the points associated with the organ that serves to health, balance, production, and reproduction in livestock [5]. In the poultry, acupuncture point that stimulates female reproductive organ was located in the dorsal region joint between last thoracic and first lumbar [5]. It was expected that stimulating the same point in male ducks would give the same effect that eventually might contribute in fulfilling the need for duck breeding stock. Therefore, this study was conducted to evaluate the effect of laser puncture at ova point on the number of Sertoli and Leydig cells.

2. Materials and methods

Eighteen, five-month-old male Mojosari ducks were used in this study. Ducks were kept separately in battery cages (one cage for one duck) and fed finisher phase feed twice a day (morning and afternoon). Vitamins were supplemented to keep the immune system.

Ducks were randomly divided into three, control group (P_0), and treatment groups wherein the ducks were shot with laser dose of 0.2 Joule (P1) and 0.4 Joule (P2) at the point of reproduction. Laser acupuncture treatments were conducted using automatic 20 mW semiconductor Laser acupuncture equipment with adjustable power and dosage. Treatments were carried out for one month with an interval of three days.

2.1. Laser Acupuncture Treatment Procedure

Ducks were restrained in standing position with proper handling to avoid the ducks from moving. Shoots were aimed at one point, namely point ova which was located between the last thoracic and first lumbar joints. Correct point for the shooting was obtained by palpating the poultry spine and seeking the dorsal region joint between the last thoracic with first lumbar. The machine was turned on and set the laser beam dose. The laser beam was fired by pressing the button once and the machine would switch off automatically when the required time for each dose had been reached.

2.2. Sertoli and Leydig Cells Counting

After going through the treatments, on day-31 all ducks were slaughtered and dissected open the abdominal cavity to collect the testes. Testes were then stored in plastic pots containing 10% formalin and fixed for 48 hours. After that HE stained testes histological slides were prepared. Sertoli and Leydig cell counting were conducted under microscope with 400x magnification. The number of Sertoli and Leydig cells in each group was counted from both left and right testes of the ducks [6].

2.3. Data Analysis

Data on the number of Sertoli and Leydig cells were presented as means ± SDand difference among groups were analyzed using ANOVA followed by Duncan's multiple range test using SPSS statistical programs.

3. Results

Histological slide assessment on Leydig and Sertoli cells was carried out to observe the effect of laser acupuncture shoot on the reproductive point of Mojosari male duck. The amount of Leydig and Sertoli cells of each group are presented in **Table 1**.

On both, Leydig and Sertoli cells analysis showed significant difference which means that the laser acupuncture shoot gives a significant result ($p<0.05$). However, instead of increasing, both the number of Leydig and Sertoli cells were significantly reduced. Duncan's multiple range test analysis showed that ducks received 0.4 J shot reduced the number of Leydig and Sertoli cells more than the ducks received 0.2 J shoot and the control group. The fewest number of both cells were found in ducks received 0.4 J

Table 1: Number of Leydig and Sertoli Cells (Means ± SD) of male Mojosari ducks shot with laser acupuncture on reproduction point.

	cell number	
	Leydig	Sertoli
P_0	62.51± 2.32^a	31.48± 0.40^a
P_1	47.95± 1.90^b	23.06± 0.69^b
P_2	35.56 ± 1.85^c	12.93 ± 0.57^c

$^{a,b,and c}$ different superscript in the same column indicates a significant difference ($p<0.05$); P_0 : Control Group, ducks without laser acupuncture shoot; P_1: ducks received 0.2 J laser acupuncture shoot; P_2: ducks received 0.4 J laser acupuncture shoot; Replicates = 6; Shoots were repeated 10x with 3 days interval.

laser puncture and the highest number of both cells were found in control group (Table 1).

Light Amplification by Stimulated Emission of Radiation (Laser) basic principles are pump or energy source, medium (solid, liquid, gas, or plasma), optical feedback (cavity or resonator) [7]. The laser has been widely applied in the medical fields such as surgery, dental disease and rheumatoid arthritis [8]. Acupuncture is based on pricking a needle in and through the skin on specific places (acupuncture points) which leads to a different biological reaction in the body that can be objectively measured [9]. Mechanism of acupuncture stimulation began with the start of initial stimulation, that is laser acupuncture shooting at acupuncture point as a group of active electricity cell, which will lead to a special activity for the cells group in the form of rRNA stimulation, mRNA activation, and protein synthesis. Specific pathway connecting one point to another point toward the final terminal which is organ [4]. some studies showed that Laser acupuncture improved the vascular system, endocrine and various other body systems [10]. Laser acupuncture in action could escalate blood circulation, stimulate cell metabolism, accelerate wound healing, acute or chronic local inflammatory, accelerate the loss of swelling, pain and other inflammatory symptoms [11]. Acupuncture stimulation travel path in the female poultry reproduction which is ova point travel from nerves stimulation to the bone marrow that continues the stimulation into the brain. Brain stimulates hypothalamus to secrete GnRH that continue the stimulus into ovarium. In the male poultry, it is expected that the stimulation travel to testes with hope to stimulates the growth of Sertoli and Leydig cells. Hypothalamus secretes Gonadotropin Releasing Hormone (GnRH) to stimulate the pituitary gland to secrete Follicle Stimulating Hormone (FSH) and Luteinizing Hormone (LH). FSH role is to stimulate Sertoli cells to produce estrogen or estradiol. LH role was to stimulate Leydig cells

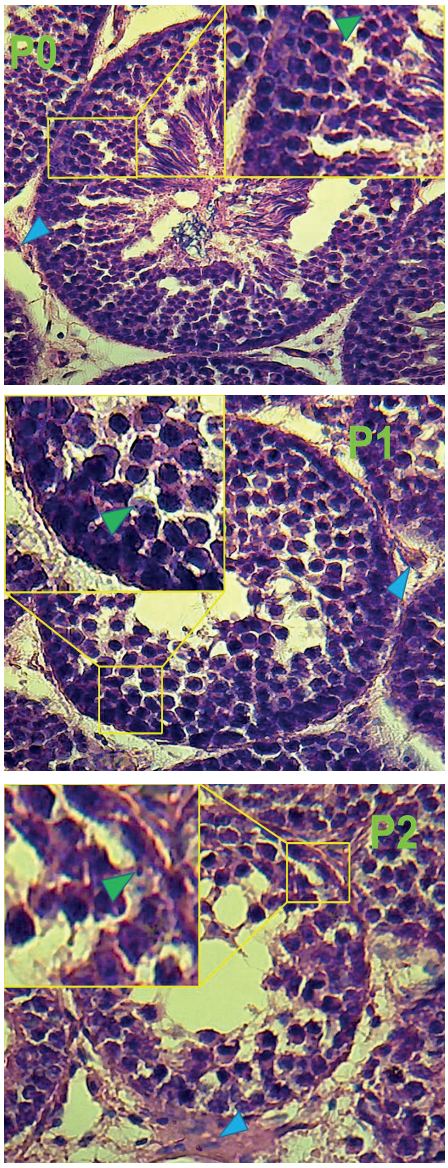

Figure 1: Seminiferous tubule of mojosari duck of each treatment (HE stain; 400x Magnification). Green arrowheads shows Sertoli cell. Light blue arrowheads shows Leydig cell. P_0: Control Group, ducks without laser acupuncture shoot; P_1: ducks received 0.2 J laser acupuncture shoot; P_2: ducks received 0.4 J laser acupuncture shoot; Replicates = 6; Shoots were repeated 10x with 3 days interval.

as the main source of testosterone in male in the presence of LH [12]. Interference with the secretion of FSH and LH can disrupt the process of spermatogenesis [13].

There are differences in the location of acupuncture point between poultry and mammals. Poultry has Bei Ji and Gu Duan point which were not found in mammals [14]. Laser acupuncture shoot on GV-4 point, in both male and female garut sheep improve reproductive function (male and female sheep reproductive acupuncture point in the same point) [4]. Meanwhile, in the poultry known that ova point which analogous to a GV-4 point in mammals improve female reproductive function in poultry [15]. This led to speculation that ova point improves male reproductive function in male

poultry because specific acupuncture point for reproduction for male poultry is still undiscovered, given that the male and females sheep reproductive point were at the same point. After laser acupuncture shoot with 0.2 J and 0.4 J dose was done, the results turn out that the number of Sertoli and Leydig cells decreased significantly. From this point can be seen that ova point which improves reproductive function in female poultry did not give the same effect in male poultry. So, it is suspected that ova point is a point specifically for female poultry not for male poultry.

Sertoli cell is a "nurse" cell of the testes which has is columnar shaped of large dimensions extending from the base of the seminiferous epithelium, the basement membrane, to the lumen of tubules [16]. Sertoli played an important role in the differentiation and development of testicular function (Depamede, 2010). The Sertoli cells also played a major role in the regulation of spermatogenesis and altering rates of spermatozoa produced [17]. Sertoli cell Nucleus is large (250-850 μm^3) and can take on several different shapes depending on the stage of seminiferous cycle and age of development [18]. The number of Sertoli cells in the testes definitely determine the testicular size and sperm production so any damage or decrease in the number of Sertoli cells will automatically reduce the sperm production and increase the number of abnormal sperm, resulting in infertility [19]. The decrease in Sertoli and Leydig cells can not be known with certainty because in the hypothesis the laser puncture shot was predicted to improve the number of both cells. It can be caused by some kind of substance or compound that inhibit the gonadotropin hormone that has a contradictive function from substance or compound that improve the male reproductive system or another hormonal factor. The decrease in Sertoli and Leydig cells supported by testicular size reduction from the control group to each treatment respectively with 0.4 J dose testes was the treatment with smallest testes size. Testicular size reduction can be caused by hormones imbalance caused from laser acupuncture shooting on ova point or laser stimulation which produce excessive energy. There is a possibility that the stimulation went to Leydig cells and the energy received by Leydig cells were too much that caused Leydig cells to rupture so testosterone levels decreased. Laser acupuncture shooting on ova point improve reproductive function in female poultry so there is a possibility that laser acupuncture on ova point improves the female reproductive hormone leading to an excessive increase in one hormone that causes other reproductive hormones to decrease. There is a possibility that testes size have a correlation with GnRH secretion and testosterone levels, as the increase in both testes size and testosterone production is ultimately under control of GnRH secretion, any association between testes size and testosterone production may instead be due to differences in the production of GnRH by hypothalamus. Testes with greater amounts

of spermatogenic tissue require, and are supplied with, higher levels of testosterone [20].

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