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Physical Conditions and Hormonal and Ovarian Profiles of Hens in Trawas, Indonesia with Egg Laying Cycle Abnormalities

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Abstract

This study evaluated the serum FSH level and the ovarian conditions of laying hens with abnormal egg-laying cycles of 38 weeks old 46 laying hens of ISA Brown strain with interrupted egg-laying cycle on an intensive laying hens production system. Based on physical observations, it was found that the weight of laying hens with an abnormal egg-laying cycle was 1.6±0.246 kg, the comb size was small and pale red, and the pubic bone was narrow. The follicles observed follicles were primordial, small white, small yellow and post ovulatory regressing follicles, but the pre-ovulatory follicles were rarely seen. The mean value of FSH was 80.71±38.97 pg/mL. Laying hens with egg-laying cycle abnormalities were characterized by altered physical conditions, low FSH level and abnormal ovarian follicular development.

Key words: Reproductive disorder, interrupted laying cycle, layer chicken, FSH, farming system.

Laying hens in Indonesia are widely reared as there is a high consumption of eggs as a source of protein. Based on data from the Central Statistics Agency (BPS, 2020), egg consumption from 1987-2017 increased by an average of 3.57% per year. Egg consumption in Indonesia reached 18.44 kg per capita per year in 2017; while it was 17.73 kg in 2018, 17.77 kg in 2019, and 28.16 kg in 2020.

Poultry farming is a significant source of revenue generation for farmers in most developing countries. It is an instrument of socio-economic change (Attia *et al.,* 2022), increasing the community's income and quality of life. The quality and quantity of egg production in laying hens can be improved by feeding, genetic selection, management practices, and disease management. However, the farmers of Trawas, East Java, Indonesia often observe abnormality in the egglaying cycles in hens during the productive period, causing economic losses. Some laying hens may never lay eggs, while others may go out of production earlier. Removal of unproductive laying hens from the flock is essential. The productivity of the hens can be assessed based on the guidelines of National 4-H Poultry Judging Manual (Clauer *et al*, 2021) for their body conditions. Hormonal factors such as FSH secreted by the pituitary gland influence the development of ovarian follicles also play an important role in egg production in poultry. This study was intended to evaluate the FSH value and the macroscopic-microscopic ovarian conditions of laying hens with abnormal egglaying cycles in Trawas, East Java, Indonesia.

Materials and Methods

Sampling

Randomly selected 46 laying hens of ISA Brown strain, aged 38 weeks with the history of egg-laying cycle disorders (rarely laying eggs)

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formed the experimental units for this study. The birds were reared in cages with standard feeding and watering managements, but without any treatment for improving production.

Weighing and evaluating the physical traits of laying hens

The weight of the birds was recorded with a digital scale, the size (small, medium, large) and color of the combs and the distance between the pubic bones (pubis bone spacing) by holding the birds by their legs were observed. The finger was gently placed between the pubic bones to determine the space between them in terms of 'finger space'.

Blood serum collection

Blood was collected from the carotid artery and jugular vein after slaughter. Approximately5 ml of blood was collected and processed for the separation of serum. Serum samples were kept frozen at-20°Cuntil analyses.

Measuring FSH value

The method used is indirect ELISA using the FSH Kit (Cat. E0504Ra, Bioassay Technology Laboratory). First, the standard solution and wash buffer were diluted, and then 50 µl of the standard solution and 50 µl of the serum sample were added to each well, covered with a sealer and incubated for 60 min at 37°C. Then, the wells were washed three times with wash buffer, and 50 µl of Substrate Solution A and 50 µl of Substrate Solution B were added to each well and incubated for 10 min at 37°C. Then,50 µl of stop solution was added to each well. The results were read using Elisa Reader on a long 450 nm wave.

Macroscopic examination of ovaries and sample collection

The laying hens were slaughtered after blood collection and abdominal necropsy was carried out to observe the presence of preovulatory follicle of 10 mm in diameter. The ovary samples of less than 0.5 cm thick were collected in a sample vial containing 10% formalin buffer for fixation for at least 24 hours.

Microscroscopic examination of ovaries

The ovary tissue sections were prepared by following standard procedure, stained with Hematoxylin Eosin (HE) and subjected to microscopic examination. Hematoxylin eosin stains cell nuclei, a purplish blue and the extracellular matrix and cytoplasm, pink in colour, with other structures taking on different shades, hues, and combinations of these colors (Eroschenko, 2017). Observations were made on the presence of Primary Follicles (PF), Small White Follicles (SWF), Small Yellow Follicles (SYF), and Post-ovulatory Regressing Follicles (RF) as described by Apperson *et al.*(2017)

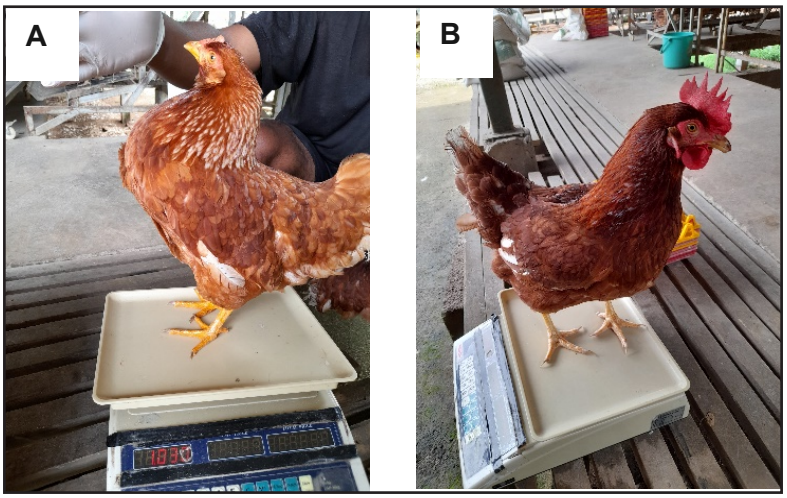

Fig. 1. Differences in comb size and color (A). Small and pale comb in hens with abnormal egglaying cycle (B). Large, well-developed, redcomb in hens with a normal egglaying cycle

Fig. 2. Difference in the pubis bone spacing (A). One finger space in hens with abnormal egglaying cycles (B). Three-finger space inhens with normal egglaying cycle.

Results and Discussion

Physical traits of laying hens and FSH value

The study found that the weight of laying hens with an abnormal egg-laying cycle was 1.6±0.246 kg, the comb size was small and pale red (Fig. 1), and the pubic bones were narrow (Fig. 2). Physical traits and FSH value of laying hens that experience egg-laying cycle abnormalities are summarized in Table I.

Ovarian condition

Macroscopic examination of ovaries revealed that the pre-ovulatory follicles (Fig. 3) in the ovaries were rarely observed(Table 2). Microscopically the follicles observed were PF, SWF and RF; whereas, larger follicles, such as SYF were rarely seen (Fig. 4).

Discussion

There is significant relationship exists between body weight and hens' reproductive indices (Pandurevic *et al.,* 2013). Body weight in poultry affects the number of eggs produced; overweight and underweight birds produce significantly fewer eggs than those with the recommended body weight (Pramanik and Chowdhury, 2021). In contrast to the weight of the laying hens in this study, which is 38 weeks, the average weight was 1.56 kg (Table I); this value was below the standard weight of laying hens reported earlier (Ray *et al*., 2022).

The small and pale combs of laying hens become physical characteristics of laying hens

Table I. Physical characters and FSH values of laying hens with egglaying cycle abnormalities (N=46)

Parameters	Value	N (%)
Body weight (Kg)	1.6±0.246	
	≤1.6	25 (54.35)
	>1.6	21 (45.65)
The combs (size and color)	Small and pale red	46 (100)
Pubic bones spacing	One finger (1.53 cm in diameter)	40 (68.96)
	≥two fingers	6 (13.04)
FSH value (pg/mL)	80.71 ± 38.97	46 (100)

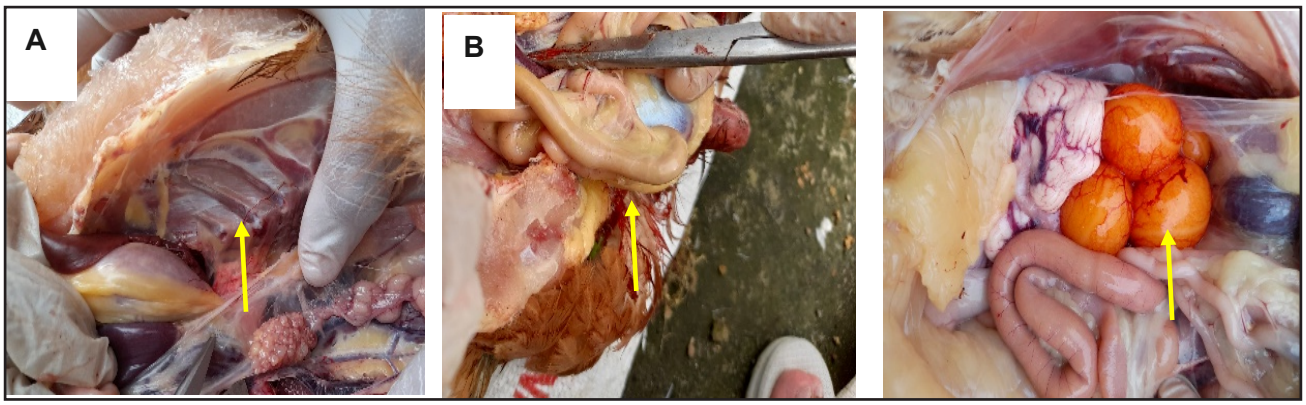

Fig. 3. Appearance of ovary. A and B: Absence of pre-ovulatory follicle;C: Presence of pre-ovulatory follicle

that rarely lay eggs. The development of the comb can be related to the level of reproductive hormones in the body (Mukhtar and Khan, 2012).Laying hens that rarely lay eggs have a pelvic bone spacing of one finger (1.53 cm distance) indicative of inactive reproductive tract of the birds.

Pre-ovulatory follicles were generally absent in hens that rarely lay eggs in the present study (Fig. 3). A total of 89.13% of the total sample had no pre-ovulatory follicles. Ovarian follicles can be divided into pre-hierarchical and hierarchical (pre-ovulatory follicles). Pre-hierarchical follicles can be divided into small white follicles (SWF), large white follicles (LWF), small yellow follicles (SYF), and large yellow follicles (LYF). Hierarchical follicles are named F1 to F6 or more according to the size of the follicles (Johnson, 2014). According to Hlokoe *et al.*(2022), the pre-ovulatory follicles are about five to six in number and are above 10 mm in diameter, with F1 being the largest follicle. Follicles of all developmental stages can be observed in reproductively active avian ovaries, ranging from larger follicles, such as SYF, SWF, PF, primordial follicles, and RF (Apperson *et al.,* 2017).

Ovarian histology observations showed that only ten samples (21.74%) had larger SYF. The absence of SYF in 78.26% of samples (Table 2) indicates incomplete follicular development tcycle. Zou *et al.,* (2020) reported that the group of Leizhou black ducks with high egg production had significantly ($P < 0.01$) more SYF compared to the group with low egg production. According to Zhou *et al*. (2022), a significant decrease in egg production in laying hens occurs due to ovarian aging, mainly caused by oxidative stress. A lack of light in poultry causes oxidative stress and severely inhibits follicular development during birds' reproduction (Cheng *et a*l., 2021).

Reproductive hormones play an essential role in follicular development with Gonado-

Table II. Macroscopic and microscopic observations of types of ovarian follicleswith egglaying cycle abnormalities (N=46)

Follicle observation	Positif (%)
Macroscopic	
Pre-ovulatory follicle	5 (10.87)
Microscopic	
PF	46 (100)
SWF	46 (100)
SYF	10 (21.74)
RF	20 (43,48)

PF=Primary Follicles; SWT= Small White Follicles; SYT= Small Yellow Follicles; RF= Post-ovulatory regressing follicle

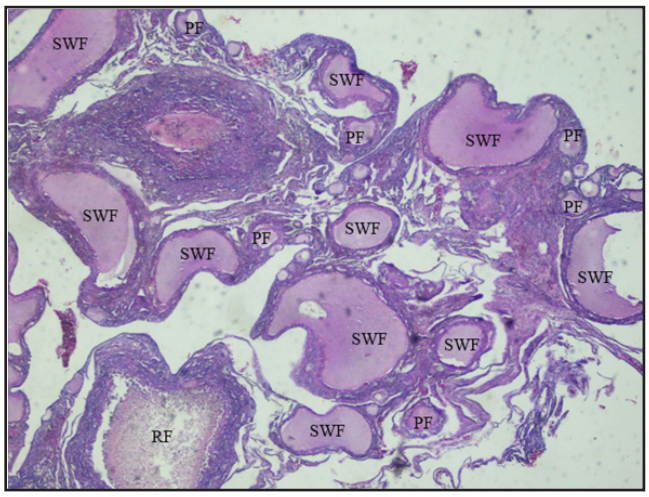

Fig. 4. The ovary of hens with egglaying cycle abnormalities. The more eosinophilic medulla of the laying hen ovary can be distinguished from the more basophilic cortex containing the follicles. This image includes small white (SWF), primary (PF), primordial (unlabeled), and post-ovulatory regressing follicles (RF) H & E. 40x.

tropin Releasing Hormone is a vital signaling factor that connects the hypothalamic-pituitary-ovarian axis to secrete gonadotropins (FSH and LH) through nerve actions that play essential roles in reproduction, including the biosynthesis of sex hormones (Lee *et al.,* 2008;Johnson, 2014; Stamatiades and Kaiser, 2018). The FSH level in laying hens is 134.77ng/mL (Cheng *et al.,* 2021); while, the birds with reproductive disorder has very low level of FSH in this study.

Conclusion

From the data obtained in this study, it can be concluded that the physical conditions, hormonal profile and the ovarian functions of hens with reproductive disorders are altered from that of a normal laying hens. More studies are required to investigate the genetic and environmental factors causing this condition.

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Conflict of interests

The authors declare that they have no competing interests.

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