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The Efficacy of Bovine Pituitary Extract for Treating Ovarian Hypofunction Cases in Bali Cattle

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ABSTRACT

Ovarian hypofunction is marked by the absence of a follicle or corpus luteum, which leads to a smooth ovarian surface during rectal examination. Furthermore, cattle affected by this disease experience an extended period of anestrus, where they do not display signs of estrus. This condition can be treated by administering gonadotropin hormones. One reliable source of these hormones is the bovine pituitary, which contains follicle-stimulating hormone (FSH) and luteinizing hormone (LH). Therefore, this study aims to demonstrate the efficacy of bovine pituitary extract as a source of FSH in enhancing follicle development and inducing estrus response in cattle with ovarian hypofunction. The study was conducted experimentally using a completely randomized design with two groups: a control group and a treatment group. In the control group, cattle were injected with a placebo (Physiological NaCl) of 1.5mL intramuscularly in two repetitions. Meanwhile, 1.5mL of bovine pituitary extract was injected into cattle intramuscularly in the treatment group. FSH levels were measured at the Integrated Biomedical Laboratory, Division of Biochemistry and Molecular Biology, Faculty of Medicine, Udayana University. The administration of treatment and observation of estrus occurrence in Bali cattle were conducted in the Livestock and Breeding Center, Sobangan Village, Mengwi Sub-district, Badung Regency, Indonesia. The results showed that the FSH level in bovine pituitary tissue extract was 189.34 mIU/mL, while the diameter of the ovarian follicle increased from 5.13±0.4mm to 10.86±0.29mm. The time of estrus occurrence was 4.56±0.81 days after the administration of the extract. We concluded that administering 0.5g of pituitary extract could increase follicle development and induce estrus in cows with ovarian hypofunction.

Key words: Bovine Pituitary Extract, Ovarian Follicle Diameter, Estrus Occurrence Time, FSH Level

INTRODUCTION

In Indonesia, the livestock sub-sector plays a crucial role in developing the agricultural sector, holding strategic value in meeting the increasing food demand due to the growing population. This is because access to an adequate supply of safe, high-quality, nutritious, and diverse food at an affordable price is essential for the community's well-being. To meet these requirements, food sources consist of plant-based (vegetables) and animal-based (livestock and fish) products. Animal-based products such as meat, eggs, and milk are the primary sources of essential nutrients, namely protein and fat. Data from the Directorate General of Livestock for the period 2009-2014 indicated an 18.2% increase in the consumption of ruminant meat from 4.4 grams/cap/day in 2009 to 5.2g/cap/day in 2014. However, during the same period, the domestic supply of local beef only fulfilled an average of 65.24% of the total national demand, resulting in the shortfall being met by imports of live cattle and frozen meat. To accelerate the increase in domestic meat production, reduce dependence on imports of meat and live cattle, as well as improve the efficiency and effectiveness of ruminant livestock farming, the Ministry of Agriculture launched the Special Efforts for Accelerating the Population of Pregnant Cattle and Buffaloes (UPSUS SIWAB) program. This program encompasses two components: increasing the population through Artificial Insemination (AI) and Natural Mating Intensification (INKA). These initiatives reflect the government's commitment to achieving self-sufficiency in

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meat production by 2026 and ensuring Indonesia's independence in meeting animal-based food needs. Simultaneously, the program aims to improve the welfare of small-scale livestock farmers. One of the critical activities to support the success of the UPSUS SIWAB program is addressing reproductive disorders in cattle.

Reproductive disorders can lead to infertility in livestock, reduced productivity, and slow population growth, as well as an increased Calving Interval (CI), culminating in economic losses for farmers (Chaurasia et al. 2010). Data from the Denpasar Veterinary Center in 2018 showed that the highest reproductive disorder cases in Bali were ovarian hypofunction (57%), followed by persistent corpus luteum (32%), endometritis (5%), ovarian cysts (2%), silent heat (2%), uterine prolapse (1%), and repeat breeding (1%). The high incidence of ovarian hypofunction significantly impacts the decline in cattle productivity (Ministry of Agriculture 2018).

Ovarian hypofunction is characterized by the absence of a follicle or corpus luteum and a smooth ovarian surface during the rectal examination (Skovorodin et al. 2022). Cattle with this condition do not exhibit signs of heat (anestrus) for an extended period. Endocrinologically, hypofunction primarily occurs due to nutritional deficiencies, preventing the anterior pituitary gland from secreting sufficient follicle-stimulating hormone (FSH) for follicle growth and development in the ovaries (Gong et al. 2020). The treatment can be accomplished by improving feed quality and administering hormone treatments, such as gonadotropins (Masruro et al. 2020; Laksmi et al. 2024). Several recent studies showed that pituitary extract can induce estrus in dairy cattle (Biancucci et al. 2016) and improve reproductive efficiency in rabbits (Sayuti et al. 2022). Two research reports demonstrated that pituitary extract and PMSG have the same effectiveness in inducing superovulation in rats (Hafizuddin et al. 2010) and cattle (Arum et al. 2013).

Despite the numerous studies on the ability of pituitary extract in reproductive management, there is a lack of data regarding FSH hormone levels in pituitary extract and its efficacy in treating ovarian hypofunction in Bali cattle. Therefore, this study aims to demonstrate the efficacy of bovine pituitary extract as a source of FSH in enhancing follicle development and inducing estrus response in cattle with ovarian hypofunction.

MATERIALS AND METHODS

This study was conducted experimentally using a Completely Randomized Design with two groups: control and treatment. In the control group, cattle were injected intramuscularly with a placebo (physiological saline) of 1.5mL. Meanwhile, a bovine pituitary extract of 1.5mL in the treatment group was injected intramuscularly with two repetitions.

The measurement of FSH hormone levels in bovine pituitary tissue was conducted at the Integrated Biomedical Laboratory of the Biochemistry and Molecular Biology Division, Faculty of Medicine, Udayana University. The administration of treatments and observation of estrus occurrence in Bali cattle were carried out at SIPADU in Sobangan Village, Mengwi Sub-district, Badung Regency. The study was conducted for three months, and 28 cattle samples with ovarian hypofunction were required.

Bovine pituitary extract was prepared using the method applied by Arum et al. (2013). Bovine pituitary glands were collected from the Pesanggaran slaughterhouse in Denpasar City, Bali, Indonesia. The collected pituitary glands were placed in a thermos and immediately transported to the laboratory for storage in a freezer until a sufficient amount was obtained. Subsequently, they were cleaned of connective tissue, separated from the outer membrane, and ground into a fine powder. About 10mL of aqua dest (water for injection) was added for every gram of pituitary tissue, and the mixture was filtered using filter paper. The resulting solution was centrifuged at 3000rpm for 20 minutes, and the supernatant was collected. The supernatant obtained from centrifugation was pituitary extract and was stored in a freezer at -20°C before use.

Estrus observations were conducted twice daily, in the morning (from 06:00 to 09:00 AM local time) and in the afternoon (from 04:00 to 06:00 PM local time). The signs of estrus observed included restlessness, swelling, and reddening of the vulva covered with transparent mucus, increased blood flow to the vaginal mucosa, accumulation of mucus-like fluid in the vagina, urination, lifting of the tail, and readiness to accept the male (Hafez and Hafez 2000).

Follicle measurements were carried out with a transrectal real-time ultrasound device (KX 5200, KAIXIN), using a transrectal transducer probe with a width of 2cm, a wavelength of 7.5 MHz, and a 3.5-meter connecting cable. The cow is restrained in a clamp cage, and then the operator, wearing gloves, performs rectal palpation to obtain palpation of the reproductive organs. The probe is lubricated with lubricant (coupling gel ultrasonography) and inserted into the rectum, guided by the operator's hand grip over the reproductive organ. Each ovary is fixed with the thumb and little finger, while the other three fingers are used to direct scanning of the surface of the ovary from lateral to medial and vice versa, several times. The internal diameter of the follicles in the ovaries is measured and recorded using ultrasonography. Follicle diameter measurements are carried out with the help of an electronic capillary on ultrasonography by measuring the length of the diameter of the horizontal line and vertical edge of the follicle and then averaging it. This study measured follicular development to Graafian follicles (Khan et al. 2015).

The data were analyzed using the Shapiro-Wilk test to determine whether the sample data were normally distributed. The Paired Sample T-test was also used to test the significance of mean differences in paired groups.

RESULTS AND DISCUSSION

The observations on follicle development, estrus occurrence time, and estrus intensity in Bali cattle commenced after administering the hypophyseal extract until the signs of estrus appeared. The measurement of FSH hormone levels was carried out using the ELISA technique. The mean diameter of ovarian follicles was measured with a specialized ultrasonography (USG) device for large animals, as shown in Table 1.

The Paired Sample T-test results showed a significant difference ($P<0.05$) in the mean diameter of ovarian follicles before and after administering the hypophyseal extract. There was an increase in the mean follicle diameter to 10.86+0.29 after the treatment. The ultrasound images showing ovarian follicles are presented in Fig. 1 and 2.

Table 1: Mean follicle diameter before and after the administration of the hypophyseal extract ($\bar{x}$+SD)

Group	Follicle Diameter (mm)	
	Before	After
Ko Control	5.12+0.21[a]	5.40+0.42[a]
Treatment	5.13+0.41[a]	10.86+0.29[b]

Note: Superscript with different letters on the same line indicates a significant difference (P<0.05).

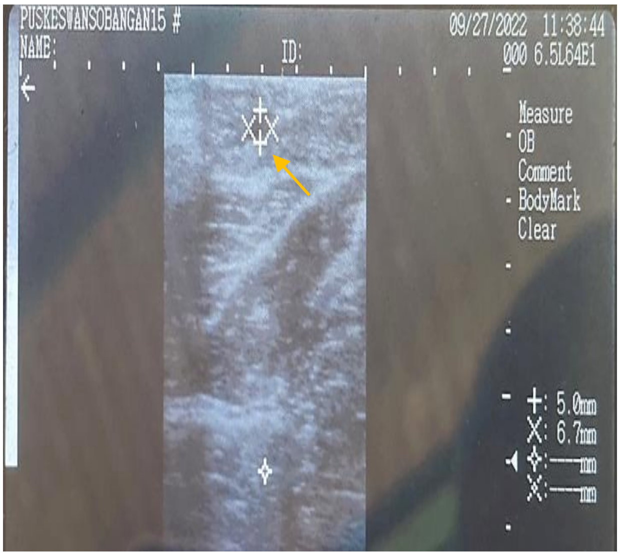

Fig. 1: Follicle diameter before treatment. The arrow indicates ovarian follicle.

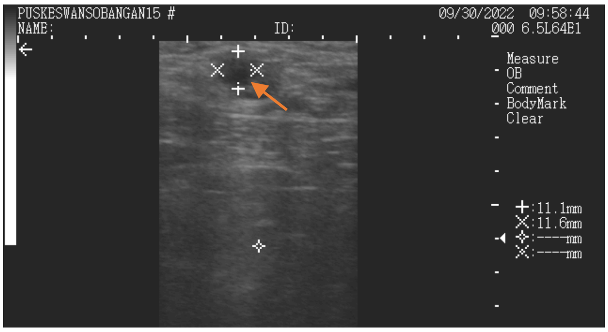

Fig. 2: Follicle diameter after treatment. The arrow indicates ovarian follicle.

Ultrasonography examination was performed twice, before the treatment and during estrus. While examining cattle's ovaries, Antral follicles appeared as fluid-filled structures on the ultrasound screen, appearing as non-echogenic black circles. On the other hand, the corpus luteum was observed as a grayish structure with uneven edges within the ovarian stroma. Ovarian follicles and corpus luteum with diameters larger than 4mm were visualized using transrectal ultrasonography, which enhanced the observation of sequential ovary changes (Fricke 2004). Widiarta et al. (2020) measured a follicle diameter of 11.06mm during estrus after administering 500µg of GnRH hormone to treat postpartum anestrus. Meanwhile, Suputra et al. (2023) reported a follicle diameter of 10.09mm at the peak of estrus after administering 50µg gonadorelin (GnRH agonist) in cattle experiencing repeated mating.

The administration of the hypophyseal extract gradually increased follicle diameter until the onset of estrus in Bali cattle with hypofunctional ovaries (Arum et al. 2013; Sayuti et al. 2022). Based on the ELISA test results, the FSH level in the hypophyseal tissue weighing 0.5g was 189.34mIU/mL. According to Hafez and Hafez (2000), the pituitary gland is located anatomically at the base of the brain and is often left behind when the brain is removed. The pituitary gland naturally produces various hormones regulating physiological functions (Al-Suhaimi and Khan 2022). Furthermore, Millar et al. (2004) emphasized the importance of the pituitary gland's position within the body for growth and reproduction. Some of the hormones produced by the pituitary gland are FSH, LH, and GH (Das et al. 2023).

The development of the follicle was stimulated by the combined actions of FSH and LH on follicular cells, with each binding to specific receptors on the surface of granulosa and theca cells (Jozkowiak et al. 2022). Activation of follicle-stimulating hormone receptor (FSHR) and LH stimulates mitosis and differentiation of granulosa and theca cells. After the preantral stage, fluid accumulates gradually in the follicle, causing its rapid enlargement. Antral follicle growth in cattle occurred at different rates during the estrus cycle and postpartum anestrus (Diskin et al. 2003). Typically, only 5 to 10 follicles grow to a diameter of 4-5mm at each stage of development. In *Bos taurus* cattle, the dominant follicle occurred when the follicle reached a diameter of 7 and 8mm (Torres et al. 2012). Ovulation was observed when the follicle reached a maximum diameter of 10.9 and 11.0mm in two-stage and three-stage development periods, respectively (Noseir 2003). Factors influencing the postpartum dominant follicle are closely related to the metabolic status of the animal, such as prepartum feeding, postpartum energy balance and parity (Rajmon et al. 2012).

The observation results of estrus occurrence in each group with the same physical status showed differences in time. The analysis showed that the mean estrus occurrence time after administering the hypophyseal extract was 4.56±0.81 days. In contrast, the control group did not exhibit estrus until the end of the study. Widiarta et al. (2020) reported that the postpartum estrus occurrence time was 4.63 days after administering the GnRH hormone. Laksmi et al. (2020) also found that the postpartum estrus occurrence time was 49.33±4.00 hours after administering the leptin hormone. According to Arum et al. (2013), bovine pituitary extract enhanced the superovulation response in Aceh cattle, as indicated by an increase in the number of corpora lutea and embryos.

Conclusion

In conclusion, this study found that administering 0.5g hypophyseal extract enhanced follicular development and induced estrus occurrence in cattle with ovarian hypofunction. estrus occurrence was attributed to increased FSH levels, which resulted in follicular development and estrogen production. the FSH level in the hypophyseal tissue weighing 0.5g was measured at 189.34 µIU/ml.

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Authors contribution

All authors contributed to the research according to their respective duties. DNDIL designed the study. IGNBT, IWS, and IWNF conducted research and data collection and evaluated ultrasound data. DNDIL and IMM performed the statistical analysis and wrote the manuscript.

Conflict of interests

All authors declared that there was no conflict of interest.

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