

Original Research Paper

# Follicle Stimulating Hormone and Gonadotropin Releasing Hormone Administration to the Superovulation of Buffalo (*Bubalus bubalis*)

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**Abstract:** This study aims to determine the response of superovulation, corpus luteum number, onset and duration of estrus in Swamp Buffalo after administration of Follicle Stimulating Hormone (FSH) and Gonadotropin Releasing Hormone (GnRH). The material used is 16 mother Swamp Buffaloes with an average body weight of 500 kg and age between 3-5 years. The dairy performance index such as the lactation period was  $127.5 \pm 104.6$  days, the milk production accounted for  $2.0 \pm 0.9$  kg/cow/day and the milk yield was  $255 \pm 209$  kg/lactation. The experimental design used was a Completely Randomized Design (CRD) consisting of four replications. Swamp buffaloes are synchronized with 5 mL of PGF $2\alpha$  injection. GnRH hormone injection on the 8th day at a dose of 500  $\mu$ g with 2 times in the morning and evening. FSH injection begins on the 10th day with 2 times a day i.e., morning and night (12 h interval) with a dose decreased for 3 days intramuscularly. Artificial insemination was carried out after signs appear estrus. The parameters observed include the superovulation response, the number of corpus luteum, the onset and duration of estrus in the Swamp Buffalo. The results obtained are the superovulation response in 56.25% Swamp Buffalo. The average number of corpus luteum, speed and duration of swamp buffalo estrus at different FSH doses and addition of 500  $\mu$ g GnRH were 1.75, 20.25 and 145.75 h respectively. It was concluded that superovulation by administering a dose of 16 ml FSH + 500  $\mu$ g GnRH has shown the best superovulation response and a long duration of estrus. The administration of 22 mL FSH dose and the addition of 500  $\mu$ g GnRH showed the fastest onset of estrus.

**Keywords:** Corpus Luteum, Superovulation Response, FSH + GnRH Hormone, Estrus, Swamp Buffalo

## Introduction

Buffalo is one of Indonesia's local livestock species that can produce meat and milk. Buffalo is one of the local livestock that is mostly raised by farmers in West Sumatra, especially in Batang Anai District, Padang Pariaman District (Afriani, 2015). Buffalo population in Padang Pariaman Regency in 2016 was recorded at 13,925 individuals, which is a significant decrease compared to the 44,226 heads recorded in 2010 (BPS, 2016). The decline in the number of the buffalo cattle population is influenced by several factors such as poor buffalo reproduction management, frequent silent heat

factors and the difficulty of estrus detection so that the implementation of artificial insemination is not appropriate (Ismaya, 2014). Productive buffalo production factors are in line with good reproductive performance (Chaiklun *et al.*, 2012). Reproductive technologies applied in Indonesia are artificial insemination, embryo transfer, estrus synchronization and superovulation (Afriani *et al.*, 2018).

Buffalos is a cattle that has an invisible cycle of estrus sometimes referred to as silent heat. This can be overcome by giving hormones that can stimulate the occurrence of estrus in buffalo cattle, so that the implementation of artificial insemination or embryo

transfer can be done to increase the buffalo population in Indonesia, especially in West Sumatra (Putro and Kusumawati, 2014). Embryo transfer procedures that can be carried out like a superovulation program with GnRH injections that aim to stimulate the formation of many follicles and mature more quickly resulting in large ovulation, so that a large number of embryos will be obtained in one ovulation cycle (Feradis, 2010; Rahman *et al.*, 2014).

Superovulation is the key to the success of TE, this can be determined by the high ovulation rate and the number of embryos obtained, while superovulation is also influenced by factors that influence the superovulation response in the donor parent, fertilization and viability of the embryo and factors related to donor parent management (Bearden *et al.*, 2003; Situmorang *et al.*, 2010). As stated at the outset of this research, this study aims to determine the response of superovulation, corpus luteum number, onset and duration of estrus in Swamp Buffalo after administration of Follicle Stimulating Hormone (FSH) and Gonadotropin Releasing Hormone (GnRH).

## Materials and Methods

### a. Donor Selection

Swamp buffaloes generally begin mating at an older age than river breeds. Young males are mainly first mated at about 3.0-3.5 years of age. Successful mating habits may go on until the animal is 12 years or even older (Ihsan, 2011).

### b. Superovulation

Estrus synchronization in buffalo was injected with PGF2 $\alpha$  hormone as much as 5 mL, GnRH hormone injection on the 8th day with a dose of 500  $\mu$ g with 2 times in the morning and evening, FSH injection was started on the 10th day with 2 times daily i.e., morning and night days (12 h interval) with a dose decreased for 3 days intra-muscularly (Rahman *et al.*, 2014). The hormone preparations used are FSH in 1 bottle of FSH (falltropin) containing 700 IU = 400 mg/ml NIH-FSH-P1. All of the donor animals were administered 16 mL (320 mg), 18 mL (360 mg), 20 mL (400 mg) and 22 mL (440 mg). On the 11th day PGF2 $\alpha$  injection was administered at a dose of 5 mL intramuscularly.

### c. Artificial Insemination

Artificial insemination was carried out for 12 h after the buffalo shows signs of estrus, in the form of symptoms and behavior of livestock. Estrus observation was carried out 3 times every day with a 12 h interval. Each donor was placed in a staple cage, artificial

insemination was done by experienced inseminators (Geres *et al.*, 2011; Miura *et al.*, 2017).

### d. Research Parameters

The parameters observed include the response rate of donor animals superovulated. Included in the parameters is the amount of Corpus Luteum (CL) after FSH administration with a dose of 16 mL (320 mg), 18 mL (360 mg), 20 mL (400 mg) and 22 mL (440 mg) and 500  $\mu$ g GnRH. Onset and duration of estrus in superovulated buffalo.

### e. Data Analysis

The data obtained were processed statistically using analysis of variance. To determine the effect of inter-treatment, further tests were performed. Further tests used were Duncan's Multiple Range Test (DMRT) according to Steel and Torrie (1995).

## Results and Discussion

### a. Superovulation Response

Superovulated donor buffalo with different FSH doses and the addition of 500  $\mu$ g GnRH obtained a total response rate of 56.25%. Of the 16 buffaloes that were given superovulation treatment, 9 donor buffaloes responded positively and 7 donor buffaloes did not respond to superovulation treatment, as shown in the Table 1.

In Table 1 above, it can be seen that in the treatment of P1 with 16 mL FSH and the addition of 500  $\mu$ g GnRH, the best response rate obtained is 75% Because the higher the percentage of responses the better the response at the first treatment or in other words, the response is calculated by dividing the amount of CL by the number of buffalo injected with that dose. It is known that buffalo is a livestock whose lust is difficult to detect, so therefore, the response rate is also difficult to reach 100% as shown in Table 1. The donor buffalo response obtained at a dose of 16 mL was lower compared to previous studies (Panjaitan, 2016) which obtained a 100% response rate in coastal cattle. Nanda (2012) reported that the use of 16 mL FSH dose obtained a response rate of 66.67%. The success of the superovulation treatment can be seen from the ovarian response measured by the amount of CL formed due to ovulation from mature follicles (de Graff). The more CL formed can be said the higher the response from the superovulation program (Afriani *et al.*, 2014).

Silva *et al.* (2009) argue that the factors that cause differences in the level of superovulation response are divided into 2 factors, namely internal and external factors. Internal factors include livestock genetics (breeds and individual sensitivity of livestock to

hormones), nutrition and reproductive organ health. External factors include the use of FSH preparations, the dose of FSH used, the season and management of the implementation in the field. Different responses can be due to the physiological factors of each different animal.

*b. The Number of Corpus Luteum (CL)*

The amount of CL in buffaloes after FSH + GnRH superovulation can be seen in Table 2.

Table 2, it can be seen that with the supplementation of various doses of the FSH hormone and the addition of 500 µg of GnRH, right ovaries are more numerous than left ovaries. This is in line with Haylan *et al.* (2009) who observe that right ovarian activity is more active at work than left ovary. Right ovary (2.261±0.4552) is longer than left ovary (2.239±0.4692). Right ovary (1.561±0.3292) is wider than left ovary (1.489±0.3989).

In Table 2, it is seen that the highest amount of CL is FSH 16 mL + 500 µg GnRH (as much as 8 CL) followed by the FSH 18 mL + 500 µg GnRH and FSH 22 mL + 500 µg GnRH (as much as 7 CL) while the lowest amount of CL is FSH 20 mL + 500 µg GnRH (as much as 6 CL). Statistical tests show that the treatment is not significantly different (P> 0.05). Syaiful (2017) reports that the use of 14 mL FSH + 200 µg GnRH provides the

best CL amount, which is 28 CL. The amount of CL superovulation in buffaloes can be seen in Table 3.

Table 3 shows that the highest amount of CL at 16 mL FSH dose + 500 µg GnRH (as much as 8 CL) followed by the acquisition of CL as much as 7 mL FSH supplementation + 500 µg GnRH and FSH 22 mL + 500 µg GnRH while the acquisition of CL as much as 7 in FSH supplementation 18 mL + 500 µg GnRH and FSH 22 mL + 500 µg GnRH while the lowest acquisition of CL is FSH 20 mL + 500 µg GnRH (as much as 6 CL). Based on statistical tests showed that the treatment was not significantly different (P> 0.05). Syaiful (2017) reports that the use of 14 ml FSH + 200 µg GnRH obtained the best CL amount, which is 28 CL. Nilchuen *et al.* (2011) reported the use of 200 mg and 250 mg FSH concentrations in Saen and Heifers crossbred cattle showing a total CL of 8.67±0.98; 7.33±0.98; 10.33±0.98 and 13.00±0.98. Ali *et al.* (2012) reported that local Bangladeshi cattle injected 320 mg FSH gave the best response to the amount of CL 13.60±0.51 while the lowest mean CL amount was 8.60±0.60 at 200 mg FSH.

*c. Onset and Duration of Estrus*

The Following Fig. 1 shows the speed and duration of estrus in buffalo.

**Table 1:** The response of Buffalo Superovulation at Different Doses of FSH and the Addition of 500 µg GnRH

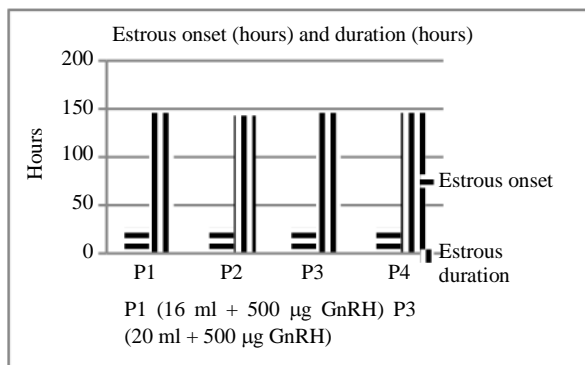
Treatment	Donor	Donor response	No response donor	Response rate (%)
P1 (16 mL + 500 µg GnRH)	4	3	1	75
P2 (18 mL + 500 µg GnRH)	4	2	2	50
P3 (20 mL + 500 µg GnRH)	4	2	2	50
P4 (22 mL + 500 µg GnRH)	4	2	3	50
Total	16	9	7	56,25

**Table 2:** Total CL Superovulation of buffaloes at different doses of FSH and the addition of 500 µg of GnRH to the right ovary and left ovary

Hormone treatment	Corpus luteum number	
	Right ovaries	Left ovaries
16 mL FSH + 500 µg GnRH	6	2
18 mL FSH + 500 µg GnRH	6	1
20 mL FSH + 500 µg GnRH	4	2
22 mL FSH + 500 µg GnRH	4	3
Total	20	8
Rate	5	2

**Table 3:** CL amount of buffalo superovulation at different doses of FSH and addition of 500 µg GnRH

Treatment	Donor #	CL #	Average
P1 (16 mL + 500 µg GnRH)	4	8,00	2,00
P2 (18 mL + 500 µg GnRH)	4	7,00	1,75
P3 (20 mL + 500 µg GnRH)	4	6,00	1,50
P4 (22 mL + 500 µg GnRH)	4	7,00	1,75
Total	16	28	1,75



**Fig. 1:** Average estrous onset and duration in swamp buffalo with different doses

Based on Fig. 1, it can be seen that the Ovsynch superovulation treatment shows that the estrus rate is not significantly different ( $P > 0.05$ ). Based on the research conducted, the results obtained estrus velocity in buffaloes ranged from 25-26 h. Yendraliza *et al.* (2012) reported that the postpartum buffalo estrus velocity in Kampar District at the level of GnRH dose synchronized with 12.5 mg of PGF2 $\alpha$  in a row in hours was  $52 \pm 6$ ;  $53.88 \pm 5.1$ ;  $27.8 \pm 2.5$ ;  $28.8 \pm 0.5$  and  $30 \pm 1.9$ . Berber *et al.* (2002) who reported that the use of one dose of GnRH can improve the estrous cycle in buffalo and the use of two doses of GnRH will accelerate the emergence of estrus in buffalo when combined with PGF2 $\alpha$ . In line with the report of Paul and Prakash (2005) who reported that the combination of the use of GnRH and PGF2 $\alpha$  would accelerate the emergence of estrus in buffalo.

Based on the results of the analysis of variance analysis. It was found that each treatment did not have a significant effect ( $P > 0.05$ ) on the duration of heat in superovulated buffaloes. The study found that the average duration of heat in buffalo is 145.75 h. The duration of estrus depends on the number and quality of different follicles. Because a large number of follicles also correlates more and more with the estrogen produced also more, it is possible that the duration of estrus produced could be longer. Control of follicular growth waves is very important in the estrus superovulation and synchronization program, which affects the duration of the estrous cycle and the length of the luteal phase (Hafez, 2008).

## Conclusion

From the research conducted, it can be concluded that the administration of 16 mL FSH dose and the addition of 500  $\mu$ g GnRH has shown the best superovulation response and long lust. The administration of a 22 mL FSH dose and the addition of 500  $\mu$ g GnRH showed the fastest heat rate.

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## Author's Contributions

**Tinda Afriyani and Ferdinal Rahim:** Research conceptualization and design.

**Tinda Afriyani, Arif Rachmat, Mangku Mundana and Anna Farhana:** Data Collection and Interpretation.

**Tinda Afriyani and Ferdinal Rahim:** Writing and Editing.

## Ethics

This research has been approved by the Committee of Ethics of the Faculty of Agriculture of Andalas University Padang, Indonesia and therefore, no ethical issues may arise after the publication of this research.

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