

# Jurnal 6

*by* Tinda Afriani

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## Effect Of GnRh Administration On Superovulation Response Of Pesisir Cows In West Sumatera

Tinda Afriani<sup>1</sup>, Endang Purwati<sup>2</sup>, James Hellyward<sup>3</sup>, Jaswandi<sup>2</sup>, Mangku Mundana<sup>1</sup>, Adisti Rstosari<sup>1</sup>, Anna Farhana<sup>2</sup>

<sup>3</sup>Department of Livestock Production, Faculty of Animal Science, Andalas University, Padang, West Sumatera, Indonesia.

<sup>2</sup>Department of Reproductive Biotechnology, Faculty of Animal Science, Andalas University, Padang, West Sumatera, Indonesia

<sup>12</sup>Department of Livestock Bussiness, Faculty of Animal Science, Andalas University, Padang, West Sumatera, Indonesia

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### Abstract

This study aimed to determine the response of superovulation in pesisir cows. Animals were treated with GnRH<sup>5</sup> at different doses, and we observed the onset, estrous duration, number of CL, and embryos. This study uses a Complete Randomized Design (CRD), with a dose of GnRH administration as a treatment. 16 pesisir cows are used in this study and divided into four groups; each cow must have been pregnant at least once. This research is performed by installing Internal Drug Release Control (CIDR) intravaginal. After the 8th day, each group was injected with GnRH 150 µg, 175 µg, 200 µg, and 225 µg, respectively. On the day – 10<sup>th</sup> amount of FSH was injected into each cow for 3 days. On the day of – 12<sup>th</sup>, cows were treated with PGF2α and started to observe estrous signs on day 13<sup>th</sup>. Artificial insemination was performed using FH (Friesian<sup>9</sup> Holstein) semen. Embryo collection was carried out on days 6 to 8 after AI was performed. Data were analyzed using analysis of variance (ANOVA), and if any significant differences were analyzed with Duncan Multiple Range Test (DMRT). The result showed that the level of GnRH administration has no significant differences in the onset and estrous duration. We also found no significant differences in the amount of CL and embryos. It was concluded that giving GnRH at different doses had no important on the onset and duration of estrous, number of CL, and the number of embryos.

**Keywords:** Corpus luteum, Embryo, Friesian Holstein, GnRH, Pesisir Cow

## Introduction

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Pesisir cattle is widely spread in West Sumatera, particularly in the Pesisir Selatan regency. Pesisir cattle are suitable in tropical areas and well adept with poor quality forage, have a proper feed conversion so pesisir cattle potentially can improve farmers income (Saladin, 1983; Hendri, 2013). The characteristics of pesisir cattle are unique; namely, the dominant body is brick red with color variations, brown and black. In addition, small body weight, short body, slender legs, small hump, and docile. Despite their small appearance and lower body weight than Bali cattle, coastal cattle are very productive, being highly adaptive to the environment in Pesisir Selatan (Bamualim et al., 2006; Wahyuni&Dewi, 2018).

Adult male pesisir cattle (aged 4 (6 years) have a bodyweight of 160 kg, lower than the bodyweight of Bali cattle (310 kg), PO cattle (388 kg), Aceh cattle (302 kg), and Madura cattle. (248kg) With a small body shape, pesisir cattle can be used as pets or pets for mini cow fans (Adrial, 2010). Pesisir cattle have a special charm in their maintenance, where there is no need for extra labor. The rearing system used by local farmers is a traditional extensive one where they are released during the day and tied up in cages at night (Zahara&Sulin, 2018). Usually, breeders provide grass when in the cage. This treatment is good because the afternoon to the morning is longer. At that time, the cattle are not doing any activity or movement so that the feed given can be optimally converted for meat production (Wahyuni&Dewi, 2018).

The population of the Pesisir cattle has been decreasing for a long time so that it can result in a decrease in the rate of growth of the cattle population (Sukarno & Setiawan, 2015). If this kind of thing is allowed to continue, it can lead to the extinction of this cattle population. This population decline is related to the traditional extensive maintenance system, high rates of productive livestock slaughter, lack of feed, lack of grazing land, and decreased genetic quality (Hendry, 2013). To increase the productivity and reproduction of pesisir cattle, it is necessary to improve the genetic quality of livestock through selection, crossbreeding with superior breeds of cattle, improving the value of feed quality, counseling ordinary breeders so as not to slaughter productive livestock, and improving maintenance management (Iskandar & Sartika, 2019).

The application of biotechnology of reproduction can improve the population of pesisir cattle, especially superovulation technology (Afriani et al., 2014). Biotechnology is a superior reproduction in reproduction to increase livestock productivity (Afriani et al., 2019). The development of biotechnology is very rapid and can be applied in technical assistance in improving the livestock population (Rege et al., 2011). Through reproductive biotechnology, technology is considered to be able to overcome challenges in the sense of increasing productivity without destroying local biological resources through efforts to overcome small-scale production constraints from farmers or the low productivity of local native livestock (Rauw et al., 1998).

There is various method to take superovulation technology; one of them is using GnRH (Gonadotropin-Releasing Hormone) hormone (Yang et al., 2011; Zumarni, 2016). Physiological basis of using GnRH to increase estrus cycle, ovulation, and fertility of female cattle (Herbert & Tigg, 2005). GnRH is naturally secreted in the hypothalamus gland, and its hormone will stimulate the pituitary gland to release follicle-stimulating hormone (FSH) and luteinizing hormone (LH),

both of those hormones are essential to stimulate follicle maturation and ovulation (Hamburg, 2014). The number of corpus luteum that develops in the ovaries after hormone injection gives an idea of the success of superovulation in cattle. The more corpus luteum is formed in the ovary, the higher the success rate of superovulation (Bartlewski et al., 2016).

Administration of GnRH is well known used to induce the estrous cycle. Injection 250 µg of GnRH twice a time can influence the estrous process more effectively than a single injection of 500 µg of GnRH (Rahman et al., 2014). However, applying a high dose of GnRH several times need more effort and cost. The ov-synch method is one solution we can take to overcome that problem. This method used PGF2 $\alpha$  to lysis the CL and followed GnRH administration (Ismaya, 2014; Afriani, 2015).

GnRH administration will stimulate superovulation. Successful superovulation is determined by the rate of ovulation and amounts of embryos acquired. To obtain a high ovulation rate, we must investigate the effect of dose GnRH administration and observe the individual response of donor and management of donor (Afriani et al., 2019).

The ability to determine the estrous cycle can improve individual response to GnRH. The occurrence of follicular waves, which occur in the mid-luteal phase, about days 9<sup>th</sup> to 12<sup>th</sup>, refers to the estrous cycle length of the Pesisir cow. Days 9<sup>th</sup> until 12<sup>th</sup> is considered appropriate days to take a superovulation program.

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## Materials and Methods

The sample used in this study is 16 Pesisir cows aged 3-5 years, have good health and healthy reproductive organs, and also multiparous cows. We ensure that each cow has BCS condition 2.5 – 3 with an average body weight of 200 - 300 kg; the sample is placed into four groups. The semen sample was used from 3 ejaculates from one head Friesian Holstein (FH) bull. Semen collection used an artificial vagina consisting of a sheath of thin rubber inserted into the cylinder thick rubber (Brito et al., 2002). Samples of semen were transferred to the laboratory and evaluated macroscopic (odor, color, consistency, concentration, and volume) and microscopic (motility, viability, abnormality, and membrane integrity).

## Research Methods

### Superovulation

Selected Pesisir cows are synchronized using CIDR (Control Internal Drug Release) implanted intravaginal for 7 days. The administration of various doses of FSH and GnRH in coastal cattle is shown in Table 1.

**Table 1.** Dosage of FSH and GnRH

Treatment	Doses (ml)
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P1	17 ml FSH + 150 µg GnRH
P2	17 ml FSH + 175 µg GnRH
P3	17 ml FSH + 200 µg GnRH
P4	17 ml FSH + 225 µg GnRH

On the tenth day, FSH injections were initiated twice daily in the morning and evening (12 h interval), with a dose reduction for three days intramuscularly. FSH in one bottle of FSH (falltropin), containing 700 IU = 400 mg/ml NIH-FSH-P1. The treatment dose was 17 ml (340 mg). On the 12th day, a 5 mL intramuscular injection of PGF2 was administered (Rahman et al., 2014; Afriani, 2015).

### Artificial insemination

Donor cattle were inseminated 12 hours after expressing the estrous sign using Friesian Holstein semen. Donor placed in clamp cage for minimizing error during insemination, AI program is done in 3 times, on the first-day insemination do in the morning following in evening and the second day in the morning. Each donor was injected with 1 straw was provided by BIB Singosari. Artificial insemination was done by experienced inseminators (Gereset al., 2011; Miura et al., 2017).

### Embryo harvest (flushing)

Flushing is carried out on the 6th to 8th day after IB is done when the embryo is in the uterine horn with the following steps: Donor cattle are subjected to epidural anesthesia with 4-6 ml of 2% Lidocaine to facilitate the insertion of a Foley catheter and reduce the incidence of defecation during flushing. The Foley catheter is lubricated with jelly, and its insertion is carried out in an aseptic manner until it reaches the uterine horn along the middle of the cornea. Uterine flushing is done by opening the inlet clamp and closing the outlet to 50 ml-500 ml of rinse media. The next step is the evaluation and selection of embryos.

### Data analysis

Four different GnRH concentrations were used in this study, and each treatment was repeated at least four times. The collected data were statistically analyzed using analysis of variance to determine the differences between treatments. Significant differences were defined as those with a P-value of 0.05. According to Steel and Torrie, the Multiple Range Test (DMRT) was used to determine the effectiveness of each treatment (1995).

### Results and discussion

#### 1. Onset and duration of estrous

Observations of estrous on cattle after administering the hormone PGF2 $\alpha$  on the 12th day. The average of observations can be seen in Table 1.



**Table 2.** The onset of estrous after administration of PGF2 $\alpha$  hormone

Replication	Treatment			
	P1	P2	P3	P4
1	29	28	28	30
2	28	26	30	27
3	29	29	29	29
4	27	28	31	30
Total	113	111	118	116
Average	28.25	27.75	29.50	29.00

From the study results obtained, the results of the onset of estrous in cows ranged on average between 27-29 hours. The results of statistical analysis (appendix 4) showed that the onset of estrous was no different ( $P>0.05$ ) in Pesisir cows. This is due to the presence of CL that responds to PGF2 $\alpha$  at the time of PGF2 $\alpha$  injection.

Between the onset of estrus and the end of estrus, the estrus duration is measured. A woman's age, body condition, and the type of hormone used for synchronization or induction of estrus all affect the length of her estrus cycle (Hastono, 2000). Table 3 shows that patients had to be treated for a long period of time.

**Table 3.** Long-time estrous cow after giving PGF2 $\alpha$  hormone

Replication	Treatment			
	P1	P2	P3	P4
1	44	45	44	42
2	45	47	42	45
3	43	44	43	43
4	46	43	41	42
Total	178	179	170	172
Average	44.50	44.75	42.50	43.00

Based on research conducted, the average results of the duration of estrous ranged from 43-44.75 hours. Based on the results of statistical tests of various analyses (Appendix 5) that each treatment showed no accurate different results ( $P>0.05$ ) against the duration of estrous in Pesisir cow. Long periods of superovulation due to increased estrogen concentrations after the maturation of the follicles. Ilham (2009) states that by injecting PGF2 $\alpha$  that melts CL formed due to GnRH, eventually, the follicles grow together so that estrogen is produced to occur. The primary function of the hormone estrogen is to stimulate the berahi, stimulate the onset of secondary genital

properties, maintain the female udder duct system and udder growth.

The growth of follicles, ovulation, and CL formation are all strongly influenced by the reproductive hormones that circulate in the body. The hypothalamus produces GnRH to stimulate the anterior pituitary's production of FSH and LH in response to estrogen or progesterone. The recruitment follicle growth process results in an increase in the mRNA for P450 aromatase. The dominant follicle contains high estrogen concentrations in the follicular fluid during morphological selection. Once the selection process is complete, the dominant follicle contains mRNA for gonadotropin receptors and steroid hormones.

## 2. Number of Corpus Luteum

The average amount of CL in coastal cows with a dose of FSH 17 ml + 150 µg GnRH is 3.0, the dose of FSH 17 ml + 175 µg GnRH is 4.5, the dose of FSH 17 ml + 200 µg GnRH is 4.0, the FSH dose of 17 ml + 225 µg GnRH is 3.5. The results of statistical analysis showed that the administration of FSH at doses of 17 ml + 150 µg GnRH, 17 ml + 175 µg GnRH, 17 ml + 200 µg GnRH, and 17 ml + 225 µg GnRH showed results that were not markedly different from CL acquisition in Coastal cows ( $P > 0.05$ ). This data can be seen in Table 4.

**Table 4.** Amount of CL superovulation result with various doses of GnRH

Replication	Treatment			
	P1	P2	P3	P4
1	1	8	6	3
2	3	4	5	4
3	4	3	2	3
4	4	3	3	4
Total	12	18	16	14
Average	3.0	4.5	4.0	3.5

CL examinations can be performed on disperovated livestock using rectal palpation or ultrasound. Rectal palpation became the primary method of CL examination in this study; ultrasound was not used. Functional CL is palpable because it protrudes from the ovary's surface, except when it has reached its maximum size, and minor functional parts appear and are not palpable during palpation (Maidaswar, 2007).

## 3. Number of Pesisir Cow Embryos

The embryo collection is carried out on the 6th day after AI is performed when the embryo is already in the uteri kornua. This data can be seen in Table 5.

**Table 5.** Number of embryos resulting from the superovulation program

Deuteronomy	Treatment			
	P1	P2	P3	P4
1	1	3	4	2
2	3	3	4	3
3	4	2	2	2
4	3	3	2	4
Total	11	13	12	11
Average	2.75	3.25	3.00	2.75

Table 5 shows the average number of embryos in each treatment is 2.75, 3.25, 3.00, and 2.75. From the analysis that has been done, it is known that the administration of various levels of GnRH exerts a no different influence ( $P>0.05$ ) on embryo acquisition in coastal cows. The largest number of embryos is obtained at the dose of FSH 17 ml + 175  $\mu$ g GnRH, while in other doses, the number of embryos is lower.

The pre-ovulatory LH surge is thought to be increased when cows in estrus are given GnRH right away. Increased LH levels help mature oocytes, induce ovulation, and improve corpus luteum function. This is in line with Shephard et al. (2014), who believe that giving GnRH will improve corpus luteum function, increase progesterone levels, and prevent premature embryonic death. One possible cause is that the cow is in estrus but does not ovulate or is anovulatory; another possibility is that the cow is ovulating, but the corpus luteum function of pregnancy is not optimal. Fertilization will fail, or the embryo will die prematurely due to this. Giving GnRH to a cow shortly after insemination was thought to induce ovulation, allowing fertilization to take place, preventing premature embryonic death, and ultimately increasing the pregnancy rate.

Giving GnRH resulted in a reasonably high pregnancy rate of around 60%. When given in the early luteal phase B, GnRH helps trigger the release of the LH hormone from the anterior pituitary, which helps to optimize the development of the corpus luteum and raise progesterone levels in the blood. Increased levels of progesterone in the blood make zygote development more likely, as well as the ability to maintain pregnancy (Lopez & Garcia, 2020). Giving GnRH to cows after ovulation acts as an indirect luteotropic, promoting the development of the corpus luteum and preventing early embryonic death.

### Conclusion

The administration of various levels of the hormone GnRH does not affect the onset and duration of estrous, the amount of CL, and the number of embryos produced. For the use of GnRH and FSH hormones with good results can be used the lowest dose of 17 ml FSH + 150  $\mu$ g GnRH. For the number of CL and the number of embryos, the best-used amount of 17 ml FSH + 175  $\mu$ g GnRH. The number of CL and the number of embryos are best-used doses of 17 ml FSH + 175  $\mu$ g GnRH.



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