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# Pesisir cattle superovulation with various dosage of Follicle Stimulating Hormone (FSH) on embryo production

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**Abstract.** This study aimed to determine the use of various doses of FSH hormone, the number of luteum corpus and the number and quality of embryos produced in the superovulation program in Pesisir cattle. The experimental design was Analysis of Variance (Anova) in a completely randomized design. The research material used 16 cows from Pesisir cattle that had been synchronized with estrous with CIDR implants for 13 days and were injected with the PGF2 $\alpha$  hormone after the release of CIDR, then scheduled AI was performed. The dose of FSH hormone was 16 ml, 17 ml, 18 ml and 19 ml, each treatment consisting of 4 replications. The injection of the FSH hormone was carried out for 3 days at a decreased dose. Collection of donor embryos was carried out on days 6 to 8 after the scheduled AI. The parameters observed included superovulation response, number of corpus luteum, number of embryos and quality of embryos. The results showed that the response of Pesisir cattle superovulation was 81.25%. The average number of CL obtained was 13.75 and embryos for Pesisir cattle was 11.25. The results also showed that the quality of the embryos obtained were grade A 19, grade B 18, grade C 7, grade D 1. It can be concluded that the use of various doses of FSH hormone at doses of 16 mg, 17 ml, 18 ml and 19 ml had shown a superovulatory response. The 17 ml dose of FSH hormone showed a large number of embryos and had the best embryo quality.

**Keywords:** Superovulatory, Corpus luteum, Number of embryos, Quality of embryos, Pesisir cattle

## 1. Introduction

Pesisir cattle is the local cattle in West Sumatra that become Indonesian germplasm and a specific superior commodity for the South Pesisir District, West Sumatera through the Decree of the Minister of Agriculture No. 2908 / Kpts / OT.140 / 6/2011 [6]. Some of the excellences of this Pesisir cattle are that they can adapt to tropical climates, are able to adapt to low nutritional value feeds, and are relatively resistant to parasites and disease [2]. The case that is often encountered in the community and Pesisir cattle breeders is the condition of the livestock that is experiencing silent heat. It can result in difficulty in detecting proper estrous that may lead to failure of fertilization during marriage [7][23].

*Silent heat* is an event that has an impact on reproductive failure so that it will affect the conditions for livestock development and a declining population. This problem can be overcome by the application of livestock reproduction biotechnology such as ET (Embryo Transfer) as an effort to increase the livestock population [3] [6]. The ET process is one of the leading technologies in the field of



reproduction to improve the genetic quality of livestock (productivity and livestock population) that can be used in livestock breeding programs by utilizing superior female and male parent seeds converensively to increase livestock productivity and population [21].

One of the program that may help the ET process is the superovulation program that functions to obtain excess eggs in one ovulation. In Pesisir cattle, females are normally able to produce one or two eggs per ovulation in one. Cycles, in contrast to males, are able to produce millions or even several billion sperm per day per male livestock. This superovulation treatment was able to obtain a higher number of egg cells [10]. This superovulation program can occur naturally and artificially. The natural process can produce twins if the egg is fertilized, but it is artificially induced by giving hormones such as Pregnant Mare Serum Gonadotropin (PMSG), Follicle Stimulating Hormone (FSH) [18][19].

Each use of the FSH and PMSG hormone applications has varying advantages. The PMSG hormone has the potential to stimulate ovarian function, but it must be used for long time to induce follicular growth without ovulation. While FSH only uses a short time that is about 2-5 hours, so that the use of FSH for superovulation can be done several times [12][17]. Some of the use of FSH that can be given to donor livestock will be influenced by the response of the individual livestock. The use of FSH in a superovulation program can stimulate follicle growth and increase the number of adult follicles in the ovaries. This study was conducted to determine the use of various FSH doses in Pesisir cattle in West Sumatra through the application of embryo transfer reproductive biotechnology to the superovulation program.

## 2. Materials and Methods

### 2.1 Research materials

The livestock used in the study were 16 cows from Pesisir cattle aged 3-5 years, normal heat cycle based on observations of 2 times of heat and had no history of reproductive disease, provision of frozen semen from Simental cows. The breeding was carried out using the Artificial Insemination method. The use of feed was given as a whole with a percentage of forage as much as 70% and concentrate as much as 30%.

*2.1.1 Research tools and material.* CIDR (*Control Internal Drug Releas*), and PGF<sub>2α</sub> hormone. Flushing media such as *lactat ringer*, antibiotic (Gentamycin G1397<sup>®</sup>, Sigma Aldrich inc), 5% calf serum, petridish, micropipette, pastuere pipette, straw, bunsen burner, eppendroff microtube size (1.5 ml; 0.5 ml and 0.2 ml), vortex, microcentrifuge, PCR machine, observation camera of gels electrophoresis result, petridish, wipes, cotton, aluminium foil, stereo microscope, foley catheter, pasteure pipette, rubber tube, needle, embryo filter, stir bars, gun AI set, CIDR applicator.

### 2.2 Research procedure

*2.2.1 Donor selection.* Cows were examined for ovaries by rectal palpation to ensure that cows have normal reproductive organs, were not pregnant and had a healthy body.

*2.2.2 Heat synchronization.* Synchronization technology in Pesisir cattle using the application of CIDR (*Control Internal Drug Release*) in the vagina for 14 days. On the 14th day the CIDR was removed and an injection of PGF<sub>2α</sub> was carried out at 5 ml intra-muscularly dose [13].

*2.2.2.1 Superovulasi.* FSH injection was carried out at the beginning of the 12<sup>th</sup> day 2 times a day, namely morning and evening (12 hour interval) with decreased doses for 3 days intra-muscularly [14]. The hormone preparations used were FSH in 1 bottle of FSH (falltropin) containing 700 IU = 400 mg / ml NIH-FSH-P1, with doses of 16 ml, 17 ml, 18 ml, and 19 ml.

*2.2.2.2 Artificial insemination.* Donors at AI in 12 hours after showing signs of heat, it was done 3 times, namely on the first day in the morning and evening and on the second day in the morning. Each donor was injected 1 straw 1 times AI. In order to facilitate the process of AI, Pesisir cattle were placed in clamp pens [1] [13].

*2.2.2.3 Embryo harvest (flushing).* After estrous or after the first AI. The female donor that will be flushed was placed in a clamp cage, then epidural anesthesia was carried out using 2 - 5 ml of 2% Lidocain Chloride in the space between the vertebrae then the feces were removed from the rectum until it is empty [5].

*2.2.2.4 Embryo quality.* The embryo evaluation was carried out under a stereo microscope at a magnification of more than 40 times. The quality of the embryos was evaluated by identification through embryo morphological examination.

### *2.3 Observed variables.*

The parameters observed included: Response rate of superovulated donor livestock. The amount of Corpus Luteum (CL) according to the FSH dose treatment. The number of CL in the right ovary and left ovary, the count was done by palpation. All embryos were collected after embryo harvest and viewed under a stereo microscope. The number of embryos and quality of embryos collected in Pesisir cattle that were superovulated according to the FSH dose treatment.

### *2.4 Data analysis*

Data processing using RAL (Completely Randomized Design) according to [20].

## **3. Results and discussion**

### *3.1 Respon Superovulasi*

The results of this study showed that the superovulated donor cow with different doses obtained a response rate of 81.25%, it can be seen in Table 1.

**Table 1.** Percentage of donor response to superovulation.

FSH Doses (ml)	Donor (number)	Donor Response (number)	Donor Non Response	Response Superovulation (%)
16	4	3	1	75
17	4	4	0	100
18	4	2	2	50
19	4	4	0	100
Total	16	13	3	81,25

Giving FSH at various dosage levels showed a different response in each cow. Giving 17 ml of FSH to 4 cows and 19 ml to 4 cows showed a positive response as the number of CL was more than 1, but at 16 ml FSH only 3 cows gave positive responses from 4 cow on injection. Meanwhile, at a dose of 18 ml, only 2 cows gave a positive response from the 4 cows that were injected. Inadequate nutrition of donor livestock and the presence of reproductive organ disorders can reduce the superovulatory response [22][8].

### *3.2 The number of corpus luteum*

The average number of CL in Pesisir cattle for a dose of 16 ml was 3.5, for a dose of 17 ml was 4 and at a dose of 18 ml was 2.75, and at a dose of 19 ml was 3.5, it can be seen in Table 2.

**Table 2.** Average number of CL in various FSH doses of Pesisir cattle.

Trial	Treatment			
	16 ml	17 ml	18 ml	19 ml
1	1	3	3	3
2	3	8	6	3
3	4	2	1	4
4	6	3	1	4
Total	14	16	11	14
Average	3.5	4	2.75	3.5

The results of the statistical analysis showed that the administration of FSH at doses of 16 ml, 17 ml, 18 ml and 19 ml showed no significant difference to the acquisition of CL in Pesisir cattle ( $P > 0.05$ ). CL examination in superovulated cow can be done by rectal palpation or by using ultrasound. In this study, rectal palpation becomes mainstay in CL examination, there has been no examination using ultrasound [16][24].

### 3.3 The number of embryos

The number of embryos after being collected on the 7<sup>th</sup> day after AI when the embryos have entered the uterine cornua, it can be seen in Table 3.

**Table 3.** The average number of embryos in various doses of FSH at Pesisir cattle.

Trial	Treatment			
	16 ml	17 ml	18 ml	19 ml
1	1	3	3	2
2	3	6	4	2
3	4	1	1	4
4	4	3	1	3
Total	12	13	9	11
Average	3	3.25	2.25	2.75

Table 3 shows the average number of embryos at the FSH doses of 16 ml, 17 ml, 18 ml and 19 ml, respectively, were 3, 3.25, 2.25 and 2.75. From the analysis, it is known that the administration of various levels of FSH doses had no significant effect ( $P > 0.05$ ) on the acquisition of embryos in Pesisir cattle. Giving FSH at a dose of 17 mg showed the highest number of embryos obtained compared to the other 3 FSH dose levels [15]. Embryos can be harvested and collected from both the fallopian tubes and from the uterus. In accordance with the objectives of ET, embryos can be harvested at a certain stage and their location can be localized based on the age of the embryo [21].

### 3.4 Quality of embryo

After evaluating the harvested embryos, it is known that the total of embryos can be seen in Table 4.

**Table 4.** Quality of embryo of Pesisir cattle.

			Quality				Total
			A	B	C	D	
Dose	16 ml	Count	4	4	3	1	12
		Expected Count	5.1	4.8	1.9	.3	12.0
	17 ml	Count	9	2	2	0	13
		Expected Count	5.5	5.2	2.0	.3	13.0
	18 ml	Count	2	6	1	0	9
		Expected Count	3.8	3.6	1.4	.2	9.0
	19 ml	Count	4	6	1	0	11
		Expected Count	4.6	4.4	1.7	.2	11.0
Total		Count	19	18	7	1	45
		Expected Count	19.0	18.0	7.0	1.0	45.0

From the results of the evaluation on the harvested , it can conclude that the dose of 17 mg resulted the highest quality of embryos of 13 embryos with grade A, then the least produced embryos was with a dose of 18 mg a of grade B.

#### 4. Conclusion

Superovulation response in Pesisir cattle was 81.25%. Superovulation at various doses of FSH obtained no different results in the acquisition of CL. Superovulation at various doses of FSH gave no different results in embryo acquisition. The administration of 16 ml, 17 ml, 18 ml and 19 ml FSH doses to Pesisir cattle showed the results of a superovulation response. At the The highest number of embryos was obtained in 17 ml FSH dose, and the resulting quality gave the best embryo grade.

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