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Abstract. This study investigates the effect of FSH supplementation on the resulting number and quality of super-ovulated Pesisir cattle embryos. This study aims investigates the effect of FSH supplementation on the resulting number and quality of super-ovulated Pesisir cattle embryos. After progesterone therapy fifteen Pesisir cattle were each given five injections of FSH followed by AI with Simmental semen. Embryos were flushed out on the sixth to eighth day after AI and counted and graded under a microscope. Cows given 16 ml and 12 ml of FSH produced the highest percentage of A quality embryos (22% and 20% respectively) while no high-quality embryos were collected from those given 14 ml FSH. 12 ml FSH treatment resulted in the highest number of B quality embryos (20%) while a14 ml dose produced less than 7%. One-third of the embryos resulting from 14 ml FSH were C grade but 12 and 16 ml dosages resulted in no embryos of this grade. The highest numbers of degraded embryos were found from cows given 12 ml (60%), while the lowest from cows given 14 ml (33.33%). The percentage of the recovered ovum that remained unfertilized from cows treated with 16 ml, 14 ml, and 12 ml were 33.3%, 66.7%, and 0% respectively. While 14ml dosages of FSH resulted in the highest number of embryos, the higher embryo from a 16 ml FSH suggests that this dosage will produce superior results.
Keywords – FSH, superovulation, embryos, Pesisir Cattle.

1. Introduction

Pesisir cattle are a local Indonesian cattle breed raised in West Sumatera, especially in South Pesisir district, in coastal areas including Padang Pariaman regency, South Pesisir and Agam regencies [4].

Pesisir cattle are raised for beef [16]. These cattle have a high ability in converting low-quality feed into meat [7] and are able to adapt well to traditional low intensity raising methods and are resistant to diseases and parasites [1].

However, Pesisir cattle numbers have recently been declining sharply and livestock productivity has decreased due to limited natural resources. The grassed areas in areas that support Pesisir cattle have declined to reduce the amount of feed available and farmers have been switching to imported cattle instead [7]. However, this local breed still has great potential to produce hybrids with Simmental cattle to increase the supply of meat for community nutrition needs and provide income to farmers in South Pesisir [3].

Reproductive biotechnology, which is advancing rapidly. such as Artificial Insemination (AI) and Embryo Transfer (ET) is one way to rapidly increase the population of these cattle and increase livestock productivity. AI has already been used extensively in Pesisir cattle but ET use is still limited. ET, however, may be more effective to increase the livestock population and also provides opportunities for embryo manipulation.

Reproductive biotechnology provides a way to overcome the challenges of meeting productivity gain targets without destroying local biological resources by overcoming the constraints of small-scale production and the low productivity of local native livestock. High-quality embryos have the potential to improve livestock population numbers and improve overall genetic quality so increasing production and profitability. This study investigates the feasibility of using super-ovulation with FSH hormone to increase corpus luteum numbers and quality of embryos in Pesisir cows to pass on the genetic advantages of superior quality cows to more hybrid offspring so increasing the productivity of the population.

2. Research methods

2.1. Research materials

Research materials used were progesterone, CIDR, superovulation *Follicle Stimulating Hormone* (FSH), lubricating gel, povidone-iodine, alcohol, cotton, tissue, lactate ringer medium, physiological NaCl, gentamicin, calf serum, lidocaine, 15 coastal cattle tail, and frozen Simmental semen.

Research equipment used were Petri dishes, micropipettes, Pasteur pipettes, pipettes, a Bunsen burner, Eppendorf microtubes size (1.5 ml; 0.5 ml and 0.2 ml), vortex, micro centrifuge, PCR machine, camera to record electrophoresis gel results, , tissue, cotton, aluminum foil, stereo microscope, Foley catheter, silicone tubing and syringe, embryo filter, stirring rod, IA gun and CIDR applicator.

2.2. Super-ovulation

Fifteen Pesisir cows divided into three groups of five cows were implanted with CIDR (containing progesterone hormone) in front of the cervix for 11 days. From day 10 the nine that had produced Corpus luteum were injected with FSH in the morning and afternoon for 3 days. Each group received a different dosage of hormone: 12 ml FSH + 200 mg GnRH, 14 ml FSH + 200 mg GnRH and 16 ml FSH + 200 mg GnRH. On day 3 FSH injections were accompanied by an injection of Capriglandin for estrus detection in the morning and afternoon. Artificial Insemination (AI) was performed after visible signs of estrus. Embryo collections were performed on days 6 to 8 after AI.

2.3. Embryo flushing

On days 6-8 after AI embryos were harvested/flushed as follows; donor cattle were placed in a special cage with a 10-20 cm elevated front. Then a 2% lidocaine chloride epidural anesthesia was administered between the last sacrum and the first coccygeal bone. A cervical dilator was used to ensure clear flow of fluid from the uterus.

Next, a Foley catheter attached to an AI gun was used to flush out each cornu in turn. After the catheter was placed in the uterus the balloon was inflated with a 20ml syringe to seal off the inside of the cornua to prevent the escape of fluid or embryo into the abdominal cavity when flushing. The AI gun was removed and a Y shaped hose connected to the Foley catheter, one tube connected to the lactate ringer/antibiotic flushing media and the other into a container equipped with an embryo filter to receive the fluid flushed from the uterus.

The collection bottle was then removed along with the upper part of the filtered vial containing any filtered embryos collected, then the number and quality status of the embryo were evaluated.

2.4. Evaluation of fresh embryo

Evaluation of the embryos was performed under a stereomicroscope at 40x magnification. The embryos were counted and graded into 5 groups according to quality (grade), namely: A, B, C, Dg (*degenerated*), Uf (*unfertilized*).

3. Results and discussions

3.1. Number of embryos

The number of embryos collected at various doses of FSH and GnRH is shown in Table 1.

Table 1. FSH Dose and number of embryos in Pesisir Cows 7 days after AI.

Treatment	Number of Embryos and Ovum per Cows
12 ml FSH	1.67±0.58
14 ml FSH	5.00±3.00
16 ml FSH	3.00±1.00
Total	3.22±2.17

There was a statistically significant difference between the number of an embryo for each dose level of FSH ($P < 0.05$) with a dose of 14ml FSH producing the largest number of embryos. This large number of embryo is because supplementation of FSH and GnRH can stimulate the ovaries to grow and mature the follicles. [5] explains that the function of FSH is to stimulate follicular growth and mature de Graff follicles and together with LH stimulates estrogen release. [14] affirm that the number of embryos recovered is highly determined by the success of the super-ovulation process triggered by the FSH hormone.

Application of gonadotropin hormone at the time of follicular wave increases the super-ovulation response. This response will be higher if the hormone is administered on the day before or on the day of follicular waves than one or two days afterward.

It this study, the number of embryos was smaller than the number of corpus lutetium observed [15]. This is because not all the embryos were recovered during flushing or were not visible under the microscope. Embryos may be destroyed when the blastomeres separate from the pellucid zone. Possibly, some embryos were lost into the abdominal cavity if too much flushing fluid was inserted. [14] suggest that the low acquisition of embryos is largely determined by the success of the superovulation process by the FSH hormone. A large and hanging uterus can prevent complete closure of the catheter balloon so that the flushing fluid can seep to the other side. When too many oocytes are produced the fimbriae may fail to capture them all.

3.2. Embryo quality

Embryos were graded based on the morphology using the scale developed [14]. The results are displayed in Table 2. Both 16ml and 12 ml dosages of FSH produced similar numbers of grade A embryos but 14 ml dose resulted in none. A similar number of B grade embryos were produced with 12 ml dosage resulting in the highest number. All C grade embryos were from cows given a 14 ml dosage. The largest number of embryos was degraded.

Differences in the quality of embryo at various doses of FSH indicate that in superovulation does not produce all oocytes simultaneously hence fertilization is not simultaneous either and embryos end up at different levels of development. [4] states that variations in the stage of embryonic development within the same cycle can affect the life of the embryo. The optimal time for insemination is important because silent estrus and different cycle lengths in cattle can lead to the less optimal timing of insemination and in lower fertility rates. Unfertilized ovum may occur because the ovum is ovulated too late to be fertilised. A degraded embryo or an unfertilized oocyte may also cause a decrease in the quality of other embryos. The genetic variation of individual cows also affects the quality of the embryo [9] as does environmental influences such as nutrients obtained by livestock also affect the quality of embryos as an adequate intake of nutrients is needed to sustain the life of the embryo optimally.

Table 2. Embryo Quality of Pesisir Cattle after different FSH Dosage.

Hormone	Embryo Quality					Total
	A	B	C	Dg	Uf	
12 ml FSH	1	1	0	0	0	2
+	0	0	0	2	0	2
200 mg GnRH	0	0	0	1	0	1
Average	0.33±0.58	0.33±0.58	0±0	1.00±1.00	0±0	1.67±0.58
14 ml FSH	0	1	3	3	1	8
+	0	0	2	3	0	5
200 mg GnRH	0	0	0	2	0	2
Average	0±0	0.33±0.58	1.67±1.53	2.67±0.58	0.33±0.58	5.00±3.00
16 ml FSH	2	0	0	0	0	2
+	0	0	0	2	1	3
200 mg GnRH	0	1	0	1	2	4
Average	0.67±1.15	0.33±0.58	0±0	1.00±1.00	1.00±1.00	3.00±1.00

From this, it can be seen that the largest number of viable embryos per cow 0.22 was obtained using a 14ml dose of FSH. However, if only high-grade embryos that are likely to result in successful pregnancies after ET are considered a 16 ml dosage produced the best results with 0.33 A and B grade embryo per cow. This number may appear small but if ET is successful then a single cow can be superovulated several times a year resulting in a larger number of high-quality offspring.

4. Conclusion

The highest number of embryos per super-ovulated cow was gained with 14 ml FSH supplementation (0.67) and the lowest number with 12 ml FSH (0.22). However, with 14 ml, no A grade embryos were obtained, and a third were C grade. No clear relationship between FSH dosage and embryo quality was evident. Although a 16 ml FSH dosage resulted in fewer embryo overall (0.33) all of these were A and B grade.

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