Estrus Performance and Steroid Level of Repeat Breeding Aceh Cattle Synchronized With Pgf2α

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Abstract
The objective of this research is to determine the estrous performance and steroid level of repeat breeding in Aceh cattle. This research included 17 female Aceh cattle, aged 3-5 years, who had been pregnant at least once. The cows were divided into two groups, fertile cows (n=7) and repeat breeding cows (n=10). All cows were estrous synchronized by 5 ml PGF2α intramuscularly (LutalyseTM, Pharmacia & Upjohn Company, Pfizer Inc.) via double injection pattern, with 11 days interval. Estrous detection to measure the intensity, heat onset and heat duration after PGF2α injection was conducted twice a day at 08.00 and 16.00, each for 30 minutes. Blood collection for progesterone measurement began on the day of the last PGF2α injection, while the blood sample for estradiol was collected during standing heat. Steroid analysis was performed by enzyme linked immunosorbent assay method (ELISA). Injection of the second PGF2 in both treatment groups managed to induce estrus in 5 cows (71.4%) in fertile cow group, and 9 cows (90.0%) in repeat breeding cow group. Intensity, onset, and duration between fertile cows group vs. repeat breeding cows group were 4.0±1.0 vs. 2.9±1.1, 46.4±29.1 vs. 49.8±28.0 hours, and 59.2±34.7 vs. 64.4±42.1 hours, respectively (P>0.05). Estradiol level showed insignificant differences (P>0.05) between fertile cows (110.4±20.1 pg/ml) and repeat breeding cows (101.6±17.4 pg/ml). Progesterone level showed significant difference (P<0.05) on Day 5 after PGF2α injection, with 2.88±2.14 ng/ml (fertile cows) and 1.13±0.68 ng/ml (repeat breeding cows). Repeat breeding Aceh cattle have lower heat intensity and progesterone level on Day 5 after PGF2α injection compared to fertile cows.

Keywords
Aceh cattle — Aceh cattle — progesterone — repeat breeding

Introduction
One cause of low productivity of beef cow is the high number of repeat breeding (RB) cases. RB problem relates to cows with unobserved abnormality but more than 3 times failed conception after mating (4). RB indication in cow is 18-24 months of calving interval, > 40% conception rate, and more than 3 service per conception value (26). Generally, the cause of RB can be divided into two, fertilization failure and early embryo death (4). El-Khadrawy et al. (9) reported several causal factors of RB : genetic, bacterial infection, anatomical abnormalities, and hormonal imbalance.

RB incidence is reported to differ between livestock with the highest incidence in cows. Incidence rate in cow is reported to be 5.5-33.33% (15). RB prevalence on Aceh cattle in Pidie District reached 58.3% (34). Several physiological conditions and management as mentioned in the previous studies, are presumed to be related to high RB prevalence in Aceh cattle. These factors include low estrus intensity, especially in high temperature and humidity, relatively inadequate rearing management (31) and relatively low steroid level, especially progesterone during luteal peak (32).

Aceh cow is one of the four Indonesian local cow breeds together with Pesisir, Madura, and Bali cows. This cow is a cross breed between local cow (Bos sondaicus) and derivative zebu cow from India (Bos indicus). Aceh cow is characterized by the relatively small body performance, firm and compact with the hump on the back in male. Female has no hump though the withers area is not flat but a bit bumpy in comparison with female Bali cow. Generally, the fur of Aceh cow has two pattern colors, either brown or brick red. Most Aceh cows have horns, but 7% of them are polled. Siregar et al. (32) reported the reproduction characteristics of Aceh cow to be having three waves of follicle growth, estrous cycle average within 17.8±0.4 days, and the average length of ovulation after synchronized by PGF2α is 3.8±0.8 days.

An interesting phenomenon in Aceh cattle progesterone concentration is peak concentration, which only reaches about 1.54 ng/ml on Day 13 of the cycle (32). This concentration is relatively lower compared to progesterone peak concentration in other type of cow. In Punganur cow, peak concentration is achieved on Day 15 with progesterone concentration reaching 10.66 ng/ml (22). In Holstein cow, peak concentration is achieved on Day 14 with the concentration > 6 ng/ml (13). Aceh cattle progesterone concentration is relatively similar to that in Sahiwal cows during midluteal
phase, which was reported to be 1.94±0.22 ng/ml (21). Low progesterone concentration is possibly related to the low fertility of Aceh cattle. Mann and Lamming (19) stated that low progesterone concentration would result in low conception rate. Willard (35) added that one of the main causes of early embryonal death occurring in RB was inadequate luteal function indicated by the low progesterone concentration.

Progesterone concentration may affect several physiological processes such as ovarian follicle dynamics and uterine function. Previous researches showed that progesterone and estradiol concentration in lactating dairy cows were lower compared to non-lactating dairy cow, thus affecting ovarian follicle dynamics. Lactating dairy cattle has more abundant larger follicles, larger ovulation follicles, lower estradiol concentrations, and longer ovulation interval, which is followed by the low ability of the follicle to produce normal embryo (27). Mihm et al. (20) further added that the low progesterone concentration was a predisposition for higher pulsating frequency of luteinizing hormone (LH) stimulating the occurrence of the persistent follicle. When the persistent follicle ovulates, the oocyte reaches its maturation phase. Oocyte might be fertilized but it would be followed by an early embryonal death.

Several researches about Aceh cattle has been conducted nowadays, including researches on estrous synchronization by prostaglandin F2 alpha (PGF2α) and progesterone (30), and vaginal epithelial cell proportion during the estrous cycle (32). The relationship of estradiol concentration (5) in Aceh cattle to the estrous intensity (12) has also been reported. However, there has never been any report about the connection between the estrous performance and the steroid level in Aceh cattle with RB condition.

**Material and Methods**

**Ethical approval.** All experimental animals were approved by the Animal Ethics Committee of Faculty of Veterinary Medicine of Syiah Kuala University.

**Experimental animal.** In our research, 17 Aceh cows aged 3-5 years were used. The cows were grouped into two groups, fertile cow (n=7) and RB cow (n=10). Fertile group consisted of cows more than two months post partum, able to conceive by one-time-insemination who had two regular cycles, lower estradiol concentrations, and longer ovulation interval, which was reported to be 1.94±0.22 ng/ml (21). Low progesterone concentration is possibly related to the low fertility of Aceh cattle. Mann and Lamming (19) stated that low progesterone concentration would result in low conception rate. Willard (35) added that one of the main causes of early embryonal death occurring in RB was inadequate luteal function indicated by the low progesterone concentration.

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**Estrous synchronization.** All cows were synchronized by 5 ml PGF2α injected intramuscularly (Lutalyse™, Pharmacia & Upjohn Company, Pfizer Inc.) using double injection strategy with the interval of 11 days. Heat detection after PGF2α was done twice a day at 08.00 and 16.00 for 30 minutes. Heat observation was conducted visually and with the help of a bull. Cows with secondary and primary estrous signs such as standing heat, mounting, restlessness, red and swollen vulva, secretion of cervical mucus, and lowering appetite were scored on 0-5 scale (5 = excellent: standing heat, mounting, restless, red and swollen vulva, secretion of cervical mucus, and lowering appetite; 4 = good: standing heat, mounting, red and swollen vulva, and secretion of cervical mucus; 3 = normal: red and swollen vulva, cervical mucus secretion, and lowering appetite; 2 = fair: red and swollen vulva and lowering appetite; 1 = poor: lowering appetite; and 0 = no estrous). The criteria were based on Sonmez et al. (33).

**Blood collection and preparation.** Blood plasma for hormone measurement was taken from the jugular vein by 10 ml disposable syringe. Blood collection for progesterone measurement began from the last day of PGF2α injection, while the blood sample for estradiol measurement was taken on the peak of estrous (stay while being mounted). Sampled blood was inserted into the tubes and placed in an ice-filled container. Blood samples were brought to the laboratory and left for 30 minutes before centrifugation to remove plasma. The centrifugation was done in 2500 rpm for 15 minutes. Plasma was taken from the tube by micropipette and transferred into the microtubes. Plasma was stored in a -20°C freezer until used for the steroid analysis. Enzyme linked immunosorbent assay (ELISA) was used for hormonal analysis.

**Progesterone/estradiol concentration measurement.** A total of 25 µl of standard liquid, the sample and the control were inserted into the ELISA microplate well, and incubated for 5 minutes at room temperature. Afterwards, 200 µl progesterone/estradiol-HRP conjugate reagent was mixed in and left to incubate for 60 minutes at room temperature. The well was then rapidly shaken to get empty from the contents. Microplate was washed 3 times with 400 µl washing solution for each well. Into each well, 200 µl substrate solution was added and the plate was incubated again for 15 minutes at room temperature. Enzymatic reaction was stopped by adding 100 µl stop solution into each well. Absorbance value was detected by the ELISA reader in 10 minutes at 450 nm wavelength.

**Data analysis.** Data percentage and heat intensity were reported descriptively while the estrous onset, estrous duration, estradiol level, and progesterone level were analyzed by T-test.

**Results**

Second PGF2α injection to each treatment group managed to induce heat in 5 cows (71.4%) within fertile cow group (n=7), and 9 cows (90.0%) in RB cow group (n=10). All cows unable to show heat symptoms had progesterone level < 10 ng/ml since Day 1 until Day 5 post injection of PGF2α. Intensity, onset and duration between fertile cow group and RB cow group were as follows: 4.0±1.0 vs. 2.9±1.1, 46.4±29.1 vs. 49.8±28.0 hours, and 59.2±34.7 vs. 64.4±42.1 hours, respectively. Estradiol level showed no significant difference (P>0.05) between fertile cow (110.4±20.1 pg/ml) and RB cow (101.6±17.4 pg/ml). For progesterone measurement, no significant difference was observed (P>0.05) between Day 1 and Day 4 post-PGF2α injection, while on Day 5 post-injection, a significant difference (P<0.05) was found with 2.88±2.14 ng/ml for fertile cow group and 1.13±0.68 ng/ml for RB group. The comparison of heat and steroid level performance between fertile
cow and RB cow is shown in Table 1.

### Discussion and conclusion

Aceh cattle estrous percentage in both treatment groups upon PGF2α application was relatively good, 71.4% (fertile cow) and 90.0% (repeat breeding cow). Siregar et al. (30) stated that Aceh cattle estrous percentage upon PGF2α induction was 80%. This is relatively similar to the estrous response upon PGF2α induction in another cow, 79.45% in Ongole cow (24), 91.67% in adult FH cow and 93.02% in FH heifers (10). Difference in response in two treatment groups is assumed to be associated with either different individual responses, or other causes that cannot be explained within the scope of our research. Progesterone concentration data in Aceh cattle not in estrous before and after PGF2α application leveled at 10 ng/ml (32). Cows that failed to respond to PGF2α injection were relatively good, 71.4% (fertile cow) and 79.45% in RB cow (4.0 ± 2.40, respectively). Table 1 shows that fertile Aceh cattle heat intensity score obtained by (30, 31) is caused by the different heat intensity scoring method. Hafizuddin et al. (20) used 1-3 score range, whereas Siregar et al. (31) and Siregar et al. (30) used 0-5 score range. Statistically indifferent heat intensity between the two groups is supported by the relatively similar estradiol concentration between the two groups. Estradiol concentration during heat in fertile cow group and RB cow group is 110.4 ± 20.1 and 101.6 ± 17.4 pg/ml, respectively (P > 0.05). This result supports Lymo et al. (18) who stated that the estrous intensity was correlating with estradiol hormone concentration in blood with 0.7 correlation value. The tendency of higher estrous intensity in fertile cows compared to RB cows (4.0 ± 1.0 vs 2.9 ± 1.1) in this research cannot be correlated with the increasing estradiol concentration during heat. This statement is backed by Ramli et al. (25), who stated that the relation between estrous intensity and estradiol concentration in Aceh cattle was only 0.392. The result of this research also shows that cows with the same heat intensity happened to have relatively varying estradiol hormone level. Therefore, the occurrence of repeat breeding in Aceh cattle may not be caused by the difference in heat intensity, which is unrelated to estradiol concentration.

Estradiol concentration in Aceh cattle during estrous is relatively higher compared to estradiol concentration in other breeds, which is 25 pg/ml (10), and between 26.75 ± 8.63 to 52.91 ± 12.99 pg/ml in fresh and frozen samples (27), and 20.24 pg/ml in Punganur cow (22). Siregar et al. (33) reported the same finding. Estradiol concentrations in Aceh cattle in proestrus, estrous, metestrus, and diastrus phase respectively are 171.99 ± 11.30, 223.13 ± 9.50, 10.05 ± 98.03, and 67.37 ± 8.75 pg/ml. The same condition was found in another Indonesian local cow, the Bali cattle. Bali cattle exhibiting one follicular wave growth have estradiol concentration of 107.77 ± 55.94 pg/ml during the peak of heat (2).
Estrous onset in both groups did not show significant difference (P > 0.05). Estrous onset in fertile cow group and RB cow group was on 46.4±29.1 and 49.8±28.0 hours, respectively. Estrous onset in repeat breeding cows, according to different studies, is relatively higher compared to our research. Selvaraju et al. (28) reported estrous onset on 59.38±0.81 hours, while Khrisnakumar and Chandrashedan (16) reported even longer onset on 70.20±0.87 hours in repeat breeding cows induced by PGF2α. Estrous response in both fertile cows and normal breeding cows under PGF2α is relatively the same.

Heat duration between the two treatment groups had also failed to show significant difference (P > 0.05). Estrous duration in fertile cow and repeat breeding cow group is 59.2±34.7 and 64.4±42.1 hours, respectively. Different result was obtained by Dadarwal et al. (7), with the average estrous duration in repeat breeding cow (3.77±0.87 days) longer than in normal cow (1.0-1.5 days). Bhat and Bhat-tacharya (6) reported the same finding. Cows undergoing ovulation delay have 72.00±4.17 hours heat duration, while fertile cows have 36.89±0.62 hours duration. Aceh cattle heat duration in both groups is longer compared to Alves et al. (3), who reported estrous duration in cows induced and cows naturally on heat 12.47±0.75 and 12.41±0.76 hours, respectively. Although statistically there was no difference, if estrous duration data were to be observed in more detail, repeat breeding cow group has four cows showing extreme estrous duration. One cow has 104 hours estrus duration, one cow has 12 hours estrus duration, and two cows have 8 hours estrus duration. Singh et al. (29) reported that 63.5% of cow with repeat breeding condition exhibited prolonged estrous. This may be caused by the high concentration of progesterone, which inhibits LH surge and, in turn, becomes the causative factor of repeat breeding in cows since insemination timing turns inaccurate. Inaccurate mating time might be the cause of repeat breeding in cows with short estrous duration.

In Aceh cattle, the average progesterone concentration (ng/ml) between the two groups did not show significant difference around estrus, with the exception of Day 5 post-PGF2α injection. Concentration around estrus is relatively similar to that from the report of Siregar et al. (32), which is 0.97±0.21 ng/ml during proestrous and 0.12±0.02 ng/ml during estrous. Naik et al. (22) reported progesterone concentration during estrus of 0.43 ng/ml. The difference in progesterone concentration around estrus, especially on Day 2 post injection, and that from the other studies, is caused by the high variation of estrous interval post injection. Thus, the average progesterone level post-PGF2α injection is > 1 ng/ml.

Progesterone concentration on Day 5 post-PGF2α injection showed significant difference between the two groups. Progesterone level in RB cows (1.13±0.68 ng/ml) is lower compared to that in fertile cow (2.88±2.14 ng/ml). This result supports the conclusion from the previous research on the positive correlation between progesterone concentration post-ovulation and embryo survival rate. High progesterone concentration is correlated with higher embryo survival. Cows with low progesterone concentration post-ovulation will suffer embryonal death, which is one of the causes of repeat breeding. Low progesterone concentration in the early luteal phase will repeat from one cycle to the next (23). From the results of this research, it can be concluded that RB Aceh cows have lower heat intensity score and relatively low progesterone level in the early luteal phase.

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References

Funkcionalni estrus i razine steroida ponovnog osjemenjavanja Aceh krava sinhroniziranih sa pgf2α

Sažetak

Uvod i ciljevi

Aceh krava je jedna od četiri Indonezijske rase krava (Pesisir, Madura i Bali). Aceh rasa je nastala križanjem između lokalnih krava (Bos sondaicus) i derivata zebu krava iz Indije (Bos indicus). Cilj ovog istraživanja je da se utvrdi funkcionalni estrus i razine steroida kod ponovnog osjemenjavanja (RB) Aceh krava.

Problem ponovnog osjemenjavanja (Repeat Breeding-RB) je jedan od dominantnih uzroka pada produktivnosti Aceh goveda. Neki od faktora koji doprinose učestalom RB Aceh krava su nizak intenzitet estrusa (posebno pri visokim temperaturama i visokoj vlazi), loši uvjeti držanja i niska razina steroida, posebno progesterona, koji je relativno nizak u lutealnom vrhuncu ciklusa. Koncentracija progesterona utječe na niz fizioloških stanja, kao što su dinamika ovarijalnih folikula i funkcija maternice. Niska koncentracija progesterona utječe na povećanje frekvencije impulsa luteinizirajućeg hormona (LH) i dovodi do perzistentnog folikula. Kada perzistentni folikul ovulira, oocita je dosegla fazu sazrijevanja. Oocita može sazrijeti, no to će biti popraćeno ranom embrionalnom smrti.

Materijal i metode

U ovom istraživanju korišteno je 17 Aceh krava starosti od 3-5 godina. Krave su podijeljene u dvije grupe, fertilne krave (n = 7) i RB krave (n = 10). Fertilna grupa krava se sastojala od krava koje su bile preko dva mjeseca post partum i imale su dva redovna spolna ciklusa. RB grupa sastojala se od krava koje nisu mogle ostati gravidne nakon tri osjemenjavanja, a pokazivale su normalan spolni ciklus. Sve krave su sinhronizirane sa 5 ml PGF2 davanog intra-muskularno (Dinolytic™, Pharmacia & Upjohn Company, Pfizer Inc.) pomoću dvostrukte strategije aplikacije sa intervalom od 11 dana. Otkrivanje estrusa nakon PGF2 je rađeno dva puta dnevno u 08.00 i 16.00 sati uz 30 minuta posmatranja. Otkrivanje estrusa je provedeno vizuelno i uz pomoć bika. Krave sa sekundarnim i primarnim znakovima estrusa kao što su tjeranje, nemir, crvena i otećena vulva, izlučivanje cervikalne služi, i smanjenje apetita su bile očijenjene skalom 0-5. Uzorkovanje krvi za mjerenje progesterona vršeno je od zadnjeg dana aplikacije PGF2, dok je uzorkovanje krvi za mjerenje estradiola vršeno na vrhuncu estrusa.

Rezultati i zaključak

Druga aplikacija PGF2α za svaku tretiranu grupu je uspjela izazvati tjeranje kod 71,4% krava u fertilnoj grupi i 90,0% krava u RB grupi. Sve krave koje su pokazale znakove estrusa su imale razinu progesterona >10 ng/ml od prvog dana do 5 dana poslije aplikacije PGF2α. Intenzitet, početak i trajanje estrus fertilne i RB grupe krava iznosio je: 4,0 ± 1,0 vs 2,9 ± 1,1 (prema skali ocjenjivanja), 46,4 ± 29,1 vs 49,8 ± 28,0 i 59,2 ± 34,7 vs 64,4 ± 42,1 sati. Nivo estradiola nije pokazao značajne razlike (P > 0,05) između fertilnih krava (110,4 ± 20,1 pg/ml) i RB krava (101,6 ± 17,4 pg/ml). U svim periodima mjerenja progesterona koji je počeo od dana posljednje aplikacije PGF2α do 4 dana poslije aplikacije, nije uočena značajna razlika (P > 0,05), dok je petog dan nakon aplikacije uočena značajna razlika (P < 0,05) i iznosila je 2,88 ± 2,14 ng/ml za fertilnu grupu krava i 1,13 ± 0,68 ng/ml za RB grupu krava. Iz rezultata ovog istraživanja može se zaključiti da RB Aceh krave imaju niži intezitet tjeranja i relativno niži nivo progesterona u ranoj lutealnoj fazi u odnosu na fertilne Aceh krave.