The Effects of Super Ovulation on the Number of Corpus Luteum of Coastal Sumatera Cows (*Bos sumatranensis*)

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Abstract. The objective of this study was to observe the effects of super ovulation treatments on the number of ovulated corpus luteum. The method used in this study was experimental. The cows were programmed to experience estrous at relatively the same time (estrous synchronization) using PGF2ά. Cows were divided randomly into four treatments, each of which consisting of four heads. The number of corpus luteum and the settled follicles was collected during the study. Data were analyzed using a Latin Square design. In case there was a significant difference, Duncan test was used thereafter. Result showed that super ovulation had a significant effect on corpus luteum and settled follicles. In conclusion, the best super ovulation protocol in the first year was the combination of 4 day daily injection of 40 mg of FSH in decreasing dose plus 250 µg of GnRH given at the time of estrous, because it was able to yield greater number of corpus luteum and embryos.

Key Words: corpus luteum, follicle, super ovulation, Coastal Sumatera Cow

Introduction

Coastal Sumatera cattle (*Bos sumatranensis*) are one of many breeds of cattle distributed in different parts of Sumatra Island. Scientific data to support the development of this kind of cattle is still minimum, however, the cattle adapts very well to the local environment and has a relatively good reproductive ability. As the local germ plasm, the coastal Sumatera cattle is important to be developed via conservation and breeding.

The program of coastal Sumatra Cow breeding is constrained due to the slow calf production. Naturally, cattle are monotocus, which means that only one egg is ovulated in each heat period. The introduction of biotechnological methods of reproduction through Multiple Ovulation and Embryo Transfer (MOET) can be one solution to overcome that problem. Embryo transfer provides the following advantages, namely an easy embryos distribution among regions even countries in frozen condition, no need to transport livestock in a long distance, availability of embryo stock sellable in large quantities, and exploitation of reproductive technologies such as embryo sexing and embryo cleavage (Gordon, 1996; Pfister-Genskow et al., 2004; Lucifero et al., 2006).

On the other hand, the increasing number of ovulated eggs in each heat period can be stimulated using Follicle Stimulating Hormone (FSH) and Pregnant Mare Serum Gonadotrophin (PMSG) either intramuscular, intrauterine or intra ovaries (Bertolini et al., 2002; Adriani et al., 2003; Duggavathi et al., 2005; Gonzalez-Bulnes at al., 2004). Corpus luteum (CL) also functions to produce progesterone that is essential to maintain pregnancy. The greater the number of corpus luteum, the greater is the pregnancy probability (Gasser et al., 2006; MadNeil et al., 2006).

Exogenous gonadotropin hormones such as FSH (Duggavathi et al., 2005) and PMSG (Adriani et al., 2006; Adriani et al., 2007) have been used extensively as the preparates to produce more ovulated ova compared to natural method. However, the results are still varied (Pfister-Genskow et al., 2004; Lucifero et al., 2006.). The FSH has a short half-life, therefore it should be given repeatedly (Kaiin and Tappa, 2005). The half-life of FSH is in the range of 2-5 hours, whereas the half-life of PMSG ranges between 5 to 6 days. PMSG can be given by injection only once. Many studies have been done to extend the half-life of FSH by dissolving FSH in the polyvinylpyrrolidone (PVP) liquid. The results show that a single injection of FSH+PVP gives better response of super ovulation in cows.

On the basis of above description, this study was designed to observe the best super ovulation technique using exogenous gonadotropin hormones to produce corpus luteum in Coastal Sumatera Cow.

Materials and Methods

Four heads of 3- 6 year old Coastal Sumatera cows that had normal estrous cycles, had delivered one to two calves and never been diagnosed of reproductive disorder were used in this study. Two months before treatment, the cows were kept intensively for the uniformity of body condition. The uniformity of experimental cow conditions was enforced by feeding similar grass and concentrate given as much as 4% of body weight, dry matter basis, containing 70% TDN and 12% protein. Concentrate was given first and grass later with a frequency of 2 times a day (NRC, 2000). Cows were programmed to synchronize estrous using PGF2ά, then divided randomly into four treatments, each of which consisted of four heads as the experimental unit. Treatments were initiated on day 9 (h-0 of estrous time). Tested treatments were presented in Table 1.

The collected data in this study were the number of corpus luteum, oocytes and fertilized oocytes (embryos). The collected data were analyzed using Analysis of Variance (Anova) proceeded with Duncan Multiple Range Test (DMRT).

Results and Discussion

The use of PGF2α for estrus syncronization was successful. It was indicated by the number of cows that showed heat symptoms (100%). The results of this research was better than those reported by Sariubang and Tambing (2006), using a single dose PGF2 and repeated doses in Bali cattle that resulted in heat symptoms of as much as 71.42 and 75% respectively. However, the first appearance of estrous in this study varied on the second and third days after the treatment. The results of this study were not significantly different from those of Perry et al. (2007) who stated that the cows showed estrous symptoms about 72 hours after injection of PGF2α, and the heat in FH cows appeared 39 to 56 hours after PGF2 injection (Walsh et al., 2007). Even though the results of this study were similar to those of other, there were individual differences in the degradation ability of corpus luteum.

The success rate of super ovulation using PGF2ά hormone can be seen from the number of corpus luteum (CL) generated. The numbers of ovulated corpus luteums that were affected by super ovulation treatments were further presented in Table 2.

The number of corpus luteums generated due to super ovulation treatment on Coastal Sumatera Cows was 78, with a range of corpus luteum produced between 2 to 10 and the

Table 1. Treatments that were tested on Coastal Sumatera Cow

Treatments	Description
T-1	40 mg FSH, 2 times a day i.m., declining dozes 7, 6, 4, 3 mg for 4 days, 15 mg $PGF_{2\alpha}$ (h-12), AI 3 times (h-14, h-15)
T-2	36 mg FSH+ 30% PVP i.m. (h-9), 15 mg $PGF_{2\alpha}$(h-12), AI 3 times (h-14, h-15)
T-3	40 mg FSH, 2 time a day i.m., declining dozes 7, 6, 4, 3 for 4 days; 15 mg $PGF_{2\alpha}$ (h-12); 250 μg GnRH at estrous; AI 3 times (h-14, h-15)
T-4	36 mg FSH+ 30%PVP i.m. (h-9); 15 mg $PGF_{2\alpha}$(h-12); 250 μg GnRH at estrous; AI 3 times (h-14, h-15).

average number of corpus luteum 4.88±2.58. The results of this study were lower than that by Adriani et al. (2009) who obtained an average number of 6.80±5.42 for corpus luteum in Brahman cows. However, based on the respective treatments, the number of corpus luteum obtained from this study can be classified in the medium category (3-4.5). The results of T-1 (40 mg FSH, 2 times a day i.m., declining dozes 7, 6, 4, 3 mg for 4 days, 15 mg $PGF_{2\alpha}$ (h-12), AI 3 times (h-14, h-15), T-2 (36 mg FSH+30% PVP i.m. (h-9), 15 mg $PGF_{2\alpha}$(h-12), AI 3 times (h-14, h-15), and T-4 (36 mg FSH+ 30% PVP i.m. (h-9); 15 mg $PGF_{2\alpha}$(h-12); 250 μg GnRH at estrous; AI 3 times (h-14, h-15) belong to the medium category, and T-3 (40 mg FSH, 2 time a day i.m., declining dozes 7, 6, 4, 3 for 4 dyas; 15 mg $PGF_{2\alpha}$ (h-12); 250 μg GnRH at estrous; AI 3 times (h-14, h-15).) belong to high category (7.25±2.50). Yusuf (1990) states that super ovulation on cows in terms of the number of corpus luteum produced, is divided into three categories; high ≥ 7, medium between 3 to 6 and low -0 to 2.

The statistical analysis showed that the treatment had highly significant effect (P <0.01) on the number of corpus luteum. T-3 (40 mg FSH, 2 time a day i.m., declining dozes 7, 6, 4, 3 for 4 dyas; 15 mg $PGF_{2\alpha}$ (h-12); 250 μg GnRH at estrous; AI 3 times (h-14, h-15) was not significantly different (P> 0.05) compared to T-1 (40 mg FSH, 2 times a day i.m., declining dozes 7, 6, 4, 3 mg for 4 days, 15 mg $PGF_{2\alpha}$ (h-12), AI 3 times (h-14, h-15), but the T-3 and T-1 were significantly different (P <0.05) compared to T-2 (36 mg FSH+30% PVP i.m. (h-9), 15 mg $PGF_{2\alpha}$(h-12), AI 3 times (h-14, h-15), and T-4 (36 mg FSH+30%PVP i.m. (h-9); 15 mg $PGF_{2\alpha}$(h-12); 250 μg GnRH at estrous; AI 3 times (h-14, h-15), whereas T-2 and T-4 had no significant difference (P>0.05). This condition indicated that the best protocol of the four treatments was the T-1 and T-3, but the trend was better for T-3. The higher the use of FSH, the higher is the number of corpus luteum produced. Higher FSH dozes would provide a better stimulation on the ovaries to produce ova (Duggavathi et al., 2005; Gasser et al., 2005; Cushman et al., 2007). On the other hand, low results obtained in T-2 and T-4 was due to the short half-life of FSH and poorly functioning PVP being used.

Palpated follicles were settled follicles, which meant the follicles were not being ovulated even though under the effect of super ovulation treatments. The number of settled follicles found is presented in Table 3.

The averages of the total number of settled follicles in this research were 33 to 1.94±0.93. The averages values obtained in this study were not different than those of the research by Adriani et al. (2009) who had the number of settled follicles in Brahman crosses of 2.0±1.97. Yamamoto et al. (1995) states, the use of 30 mg of FSH+30% PVP and a single doze of

Table 2. The numbers of ovulated corpus luteum (CL) under super ovulation treatments in Coastal Sumatera Cows

Treatments	No. of cow (head)	No. of CL	Mean of CL	Range of CL
T-1	4	25	6.25±2,36[a]	3–8
T-2	4	11	2.75±0,96[b]	2–4
T-3	4	29	7.25±2,50[a]	4–10
T-4	4	13	3.25±0,96[b]	2-4
Jumlah	16	78	4.88±2,58	2-10

Values bearing different superscript at the same column differ significantly (P<0.05).

T-1: 40 mg FSH, 2 times a day i.m., declining dozes 7, 6, 4, 3 mg for 4 days, 15 mg $PGF_{2\alpha}$ (h-12), AI 3 times (h-14, h-15). **T-2**: 36 mg FSH+30% PVP i.m. (h-9), 15 mg $PGF_{2\alpha}$(h-12), AI 3 times (h-14, h-15). **T-3**: 40 mg FSH, 2 time a day i.m., declining dozes 7, 6, 4, 3 for 4 days; 15 mg $PGF_{2\alpha}$ (h-12); 250 μg GnRH at estrous; AI 3 times (h-14, h-15). **T-4**: 36 mg FSH+30% PVP i.m. (h-9); 15 mg $PGF_{2\alpha}$(h-12); 250 μg GnRH at estrous; AI 3 times (h-14, h-15).

Table 3. The number of settled follicles as affected by super ovulation treatments on Coastal Sumatera Cows

Treatments	No. of cows (head)	No. of settled folllicles	Mean of settled follicles	Cows with settled follicles (head)
T-1	4	10	2.50±1,29[a]	4
T-2	4	5	1.25±0,50[b]	4
T-3	4	10	2.50±0,58[a]	4
T-4	4	6	1.50±0,58[b]	4
Total	16	33	1.94±0,93	16

Values bearing different superscript at the same column differ significantly (P<0.05).
T-1: 40 mg FSH, 2 times a day i.m., declining dozes 7, 6, 4, 3 mg for 4 days, 15 mg $PGF_{2\alpha}$ (h-12), AI 3 times (h-14, h-15). **T-2**: 36 mg FSH+30% PVP i.m. (h-9), 15 mg $PGF_{2\alpha}$(h-12), AI 3 times (h-14, h-15). **T-3**: 40 mg FSH, 2 time a day i.m., declining dozes 7, 6, 4, 3 for 4 days; 15 mg $PGF_{2\alpha}$ (h-12); 250 μg GnRH at estrous; AI 3 times (h-14, h-15). **T-4**: 36 mg FSH+30% PVP i.m. (h-9); 15 mg $PGF_{2\alpha}$(h-12); 250 μg GnRH at estrous; AI 3 times (h-14, h-15).

FSH of 30 mg, resulted in the averages number of follicles of 3.2 and 2.3 for the first and second sequence, respectively. This condition showed that there were differences in individual response among cattle and hormone being used.

The Anova showed that the treatments affected the number of settled follicles (P<0.05). Diverse species and levels of hormones affected the number of settled follicles. The DMRT showed that T-3 (40 mg FSH, 2 time a day i.m., declining dozes 7, 6, 4, 3 for 4 dyas; 15 mg $PGF_{2\alpha}$ (h-12); 250 μg GnRH at estrous; AI 3 times (h-14, h-15) and T-1 (40 mg FSH, 2 times a day i.m., declining dozes 7, 6, 4, 3 mg for 4 days, 15 mg $PGF_{2\alpha}$ (h-12), AI 3 times (h-14, h-15) were not significantly different (P> 0.05) but the treatment T-3 and T-1 were significantly different (P <0.05) compared to T-2 (36 mg FSH+30% PVP i.m. (h-9), 15 mg $PGF_{2\alpha}$(h-12), AI 3 times (h-14, h-15), and T-4 (36 mg FSH+ 30%PVP i.m. (h-9); 15 mg $PGF_{2\alpha}$(h-12); 250 μg GnRH at estrous; AI 3 times (h-14, h-15). However, T-2 and T-4 had no significant difference (P>0.05). Based on this test, it is obvious that treatments T-1 and T-3 were the best treatments.

Conclusions

It can be concluded that the best protocol of super ovulation is the use of 40 mg of FSH, 2 times i.m a day, the declining dose 7, 6, 4, 3 for 4 days; 15 mg PGF2ά (h-12); 250 μg GnRH at estrus; IB 3 times (14 h-, h-15) that produced the greatest numbers of corpus luteum and embryo.

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