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THE EFFECTIVENESS OF hCG IN THE DYNAMICS OF FUNCTIONAL STRUCTURE OF SUPEROVULATED ACEH CATTLE OVARIES USING PREGNANT MARE SERUM GONADOTROPINS

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ABSTRACT

This study aimed to determine the biopotency of pregnant mare serum gonadotropin (PMSG) in stimulating the formation of dominant follicles and the biopotency of human chorionic gonadotropin (hCG) in triggering an increase in the ovulation rate of the natural-estrus donor group and the prostaglandin F2α (PGF2α) induced group using female Aceh cattle (n= 20) which were divided into two treatment groups: the group with natural estrus (A) and the group with PGF2α -induced estrus (B). Each donor group was superovulated using the injection of PMSG-hCG (A1, B1) and only PMSG (A2, B2). The observations on total follicles and CL were carried out at D10, D14/D+0 and D21/D+7 using ultrasonography (USG). The results showed that PMSG gave a better superovulatory response to the development of dominant follicles ($P<0.01$) at A1, A2, B1, and B2. The administration of hCG hormone 2000 IU i.m. was not able to increase the rate of ovulation. Total follicles after the addition of hCG were also not significantly different between A1:A2 and B1:B2. Statistically, the number of CL in all donor groups in D21 was not significantly different ($P>0.05$). It concluded, superovulation using PMSG gave the same results to the donor cows with natural estrus and the donor cows with PGF2α-induced estrus. The hCG hormone with a dose of 2000 IU injected i.m. was also not able to increase the ovulation rate of Aceh cattle superovulated with PMSG 2500 IU i.m.

Key words: corpus luteum, follicle, hCG, ovary, superovulation

ABSTRAK

Penelitian bertujuan menentukan biopotensi pregnant mare serum gonadotropin (PMSG) dalam menstimulasi pembentukan folikel dominan dan biopotensi human chorionic gonadotropin (hCG) dalam memicu terjadinya peningkatan laju ovulasi pada kelompok donor estrus alamiah dan yang diinduksi prostaglandin F2α (PGF2α). Dalam penelitian ini digunakan sapi aceh betina induk (n= 20) yang dibagi menjadi dua kelompok perlakuan yaitu estrus alamiah (A) dan estrus yang diinduksi PGF2α (B). Selanjutnya setiap kelompok donor dilakukan superovulasi dengan penyuntikan PMSG-hCG (A1, B1) dan hanya PMSG (A2, B2). Pengamatan terhadap total folikel dan corpus luteum (CL) dilakukan pada D10, D14/D^{+}0 dan D21/D^{+}7 menggunakan ultrasonografi (USG). Hasil penelitian menunjukkan PMSG memberikan respons superovulasi yang lebih baik, terhadap perkembangan folikel dominan ($P<0,01$) pada A1, A2, B1, B2. Pemberian hormon hCG 2000 IU secara intramuskulus belum mampu meningkatkan laju ovulasi. Total folikel setelah penambahan hCG tidak berbeda nyata pada A1:A2, B1:B2. Secara statistik jumlah CL pada semua kelompok donor di D21 tidak berbeda nyata ($P>0,05$). Disimpulkan bahwa, superovulasi menggunakan PMSG menghasilkan folikel dominan yang sama jumlahnya pada kelompok donor alamiah maupun yang diinduksi. Penyuntikan hormon hCG 2000 IU belum mampu meningkatkan laju ovulasi pada sapi aceh yang disuperovulasi dengan PMSG 2500 IU.

Kata kunci: corpus luteum, folikel, hCG, ovary, superovulation

INTRODUCTION

Aceh cattle are one of the local cattle designated as Indonesian germplasm, which is a wealth of Indonesian livestock genetic resources that need to be protected and preserved (Kepmentan 2011). However, the quality of the livestock breeds is still poor, which is likely caused by cross-breeding and unplanned crossing-breeding programs (Armansyah *et al*. 2011). For the Acehnese people, cows are very important cattle. The threat of extinction of Aceh cattle due to uncontrolled cross-breeding has a wide impact on the socio-economic life of the Acehnese (Abdullah 2008). Therefore, it is necessary to conserve Aceh cattle and increase the population rate without changing their characteristics. One of the biotechnology applications that can be used to preserve and increase the Aceh cattle population is the superovulation technique that involves embryo transfer and artificial insemination.

Superovulation is one of the biotechnology applications that greatly affects the success of embryo transfer (ET) and is expected to increase the population of Aceh cattle. In contrast to bulls which are able to produce millions and even billions of spermatozoa per day, female cows are only able to produce one or two eggs at each time of ovulation in a single estrus cycle (Supriatna 2018). The use of superovulation applications aims to stimulate the process of recruitment of large numbers of follicles to grow, develop, mature, and ovulate, thereby producing the maximum number of embryos that can be transferred (Bó and Mapletoft 2014), and increasing the number of offspring, both male and female, from donors which are genetically superior (Faizah *et al*. 2018). The success of superovulation is strongly influenced by the induction of exogenous gonadotropin hormones. Filatov *et al*. (2017) stated that to obtain a greater number of mature oocytes, all of the important factors for gonadotropins for normal follicular growth and development, such as FSH and LH, are needed.

One type of gonadotropin hormone that is often used is Pregnant Mare Serum Gonadotropin (PMSG) which is a glycoprotein hormone, consisting of subunits

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α and β, with a high potential in stimulating cow ovaries to produce follicles and corpus luteum (CL). When separated, the two subunits do not have any biological activity. However, when united, they can have the biopotency of endogenous follicle stimulating hormone (FSH) and luteinizing hormone (LH) which can stimulate biological activity in the ovaries (Papkoff 1978). PMSG hormones have activities, such as FSH and LH, that can induce the ovaries to stimulate follicle growth and maturation (Zolbin *et al*. 2018; Decourt *et al*. 2019). According to Çizmeci and Güler (2018), the recommended dose of PMSG in cattle is 2000-4000 IU, whereas Amiruddin *et al*. (2013) argued that the recommended dose is 1500-3000 IU injected intramuscularly. PMSG has a long half-life, which is 118-123 hours, which makes it have the potential to cause a negative rebound effect on the pituitary, causing LH not to be secreted (Supriatna *et al.* 1998). Ovaries that are continuously stimulated without LH secretion will produce persistent follicles.

To reduce the negative effects of residual PMSG administration, the administration of chorionic gonadotropin (hCG) hormone can be performed with an aim to prevent the normal involution of CL cells, which consequently decreases the secretion of the progesterone and increases the levels of estrogen. The hCG hormone has a biological activity similar to that of LH which can prolong the life span of the corpus luteum (Nishigai *et al*. 2001), increase the synthesis of progesterone, and induce ovulation throughout the estrus cycle (de Rensis *et al*. 2009). The timing of estrus and ovulation is influenced by the maturation of the preovulatory follicles at the time of luteolysis and the decrease in progesterone concentrations. The estrus synchronization system that uniforms the condition of the preovulatory follicles can reduce the variation of follicle maturity during luteolysis and increase the uniformity of the appearance of estrus. The research findings of Balumbi *et al*. (2019) showed that the onset of estrus was 47.55 hours to 53.28 hours after the injection of prostaglandin F2α (PGF2α). The current study thus aimed to determine the biopotency of PMSG in stimulating the formation of dominant follicles, determine the biopotency of hCG in triggering an increase in ovulation rate, and compare the potential of the group of donor cows with natural estrus and the group with PGF2α hormone-induced estrus in producing dominant follicles.

MATERIALS AND METHODS

Experimental Animals

This study used 20 female Aceh cattle (*Bos indicus*) as donors with the following criteria: aged 3-5 years, had a body weight of 200-250 kg, had given birth at least once, had good health based on the health examination results (free of disease), had intact reproductive organs, had regular cycles, were fertile and not pregnant. Donor cows were given forage feed in the form of elephant grass (*Pennisetum purpureum*) as well as a concentrate with a crude protein content of 10.02% as an additional feed source. Feed was given as much as 2-3% dry matter of body weight with a ratio of forage and concentrate feed of 60%:40%. Drinking water was provided *ad libitum.*

Research Procedure

The donor cows were divided into 2 groups (n=10), namely the group with natural estrus group (A) and the group with PGF2α hormone-induced estrus (B). Each group (n=5) received superovulation treatment in the form of PMSG induction (A1, A2, B1, and B2). Groups A1 and B1 were given additional induction in the form of hCG. Previously, the reproductive status of each donor in all treatment groups (A1, A2, B1 and B2) was checked to ensure that the studied cows were not pregnant and had normal functioning reproductive organs. The examination was performed by using rectal palpation and ultrasound equipped with a 5 MHz linear probe.

Superovulation Treatment

The estrus cycles of both donor groups were observed. The donors were placed in isolation cages to allow the observation of the onset of estrus in Group B to be performed. Prior to the superovulation treatment, Group B was synchronized using PGF2α (Estrumate, Intervet, Boxmeer, Holland) with a dose of 2 mL, i.m. for estrus synchronization. During the estrus phase, the reproductive organs were examined to determine the size of the left and right ovaries along with the presence of functional ovarian structures (follicle and CL). The superovulation treatment was carried out by injecting PMSG (Folligon™, Intervet, Boxmeer, Holland) by i.m with a dose of 2500 IU on the 10^{th} day after estrus. Then, the reproductive organs were observed prior to the superovulation (D10) by using rectal palpation or ultrasound to see the response of the ovaries. This observation was carried out by measuring the left and right ovaries and determining the number of functional ovarian structures (follicles and CL) present. On Day 12 (on the estrus cycle), PGF2α injection (Estrumate, Intervet, Boxmeer, Holland) was administered i.m. with a dose of 1 ml in the morning and evening to each treatment group (A1, A2, B1, and B2) to lyse CL. Superovulated donors would experience estrus about 42-60 hours after prostaglandin injection, namely on D14 (Supriatna 2018). Groups A1 and B1 were given additional induction of hCG (Chorullon™, Intervet, Boxmeer, Holland) by i.m. during estrus (D14 or D+0) with a dose of 2000 IU. Changes in ovarian responses and functional structure (number of follicles and CL) were observed again after the superovulation treatment, namely during estrus (D14 or D+0) and on Day 7 after estrus in the superovulation program (D21 or D+7). Follicles were classified according to their diameters, as follows: small follicles (1-4 mm), medium follicles (>4-8 mm), and large follicles (>8 mm) (Lucy *et al*. 1992).

The parameters observed and measured before the superovulation treatment were estrus intensity, number of ovarian follicles, and CL at D10. The observation of estrus intensity was carried out by observing the signs of estrus, such as standing, riding other cows, restlessness, nervousness, red and swollen vulva, cervical mucus, and decreased feed intake (Sönmez *et al*. 2005). Observations of estrus were carried out in the morning (at 06.00 WIB (UTC+7)) and in the evening (at 18.00 WIB (UTC+7) by observing the appearance of vaginal mucus that is mucous and clear hanging on the external reproductive organs. The parameters measured after the superovulation treatment were the number of ovarian follicles and CL at the time of estrus ($D^{+}0$) and on the 7^{th} day after estrus in the superovulation program ($D^{+}7$).

Data Analysis

Total follicles (based on the size classification) and total CL obtained from each treatment group were analyzed using the Analysis of Variance (ANOVA) and continue with the Duncan test (Steel and Torrie 1993) to see the difference in each treatment.

RESULTS AND DISCUSSION

Based on the observations, it was found that estrus synchronization using prostaglandin hormone in Aceh cattle gave a fairly good response; all of the 10 synchronized donors showed the signs of estrus three days after the PGF2α injection. The appearance of estrus in all donors seemed to occur because they were in the luteal phase at the time of PGF2α, injection, which was identified through the finding of functional CL by rectal palpation. Stötzel *et al*. (2012) proved that the administration of PGF2α to functional CL can cause luteolytic within a few hours, thereby reducing progesterone concentrations and stimulating the anterior pituitary to release FSH and LH. This allowed the follicles to develop as well as increased the levels of estrogen which led to estrus. The intensity of estrus is characterized by the appearance of cervical mucus, red and swollen vulva, and decreased appetite, which were clearly visible in almost all donors in the study. This estrus intensity was also shown by the donor group with natural estrus. In addition, the natural-estrus donor group also showed signs of estrus intensity by riding other cows. These conditions indicate that the reproductive status of the donors used in this study was good and the donors had a normal reproductive cycle enabling them to respond to the injected prostaglandin.

Fauzi *et al*. (2017) stated that the average time of appearance of estrus was 1-3 days after the PGF2α injection. In line with the opinion of Melia *et al*. (2013), CL started to regress the day after the PGF2α injection and finished on the third day. The provision of good quality and quantity of feed is also one of the factors that accelerate the emergence of estrus. Reith and Hoy (2018) stated that the slow appearance of estrus signs can be due to several factors, such as nutrition, livestock physiology, and environmental conditions. Meanwhile, according to Abraham (2017), the length of time in estrus can be influenced by various factors, such as breeds, climate, nutrition, stress, and cattle health conditions. Leroy *et al*. (2018) and Moore *et al*. (2021) proved that the intensity of estrus in each individual is strongly influenced by the body condition score (BCS).

Large follicles in all treatment groups before PMSG induction (D10) were not statistically significantly different ($P>0.05$) in both the natural estrus group and the PGF2α-induced estrus group. However, in the superovulation treatment after PMSG injection (D14), the mean value of large follicles in each group increased sharply (see Table 1), which was statistically very significantly different ($P<0.01$), with the mean value of A1 (1.00±1.00 to 36.40±5.44), A2 (2.20±1.10 to 41.00±14.64), B1 (1.20±1.64 to 36.40±8.75) and B2 (1.20±1.64 to 20.80±4.61). The results showed that PMSG has a good super ovulatory biopotential. The number of large follicles produced after the PMSG administration increased, indicating that PMSG had an effect on promoting follicle growth and maturation. In line with the research findings of Jitjumnong *et al*. (2019), the diameter of the follicles was larger in the group of cows with PMSG compared to the control group without the addition of PMSG. This indicates an excellent super ovulatory response. Sirjani *et al*. (2011) also mentioned that administering PMSG the day before or during controlled intravaginal drug-releasing (CIDR) extraction can promote the development of large follicles in both ovaries.

The development of follicles in one estrous cycle can be seen through the increasing size of the follicle diameter until a dominant follicle is formed and ovulation or atresia occurs (Jitjumnong *et al.* 2019). After the superovulation treatment, the number of follicles increased and the diameter of the follicles also

Table 1. Total ovarian follicles by size classification at the time of PMSG 2500 IU treatment in superovulation

Treatments	Total follicles							
	Pra SOV (D10)				Estrus (D14)			
	A1	A2	B1	B2	A1	A2	B1	B2
Small follicle (1-4 mm)	17.60±7.96	15.60±10.26	8.40±1.82	13.80±10.33	1.60±3.05	2.60±4.22	2.00±3.46	3.00±2.65
Medium follicle (>4-8 mm)	11.60±7.60	8.40±3.52	11.20±6.30	15.00±7.81	10.20±11.26	5.60±5.94	9.20±6.30	22.00±15.07
Large follicle (>8 mm)	1.00±1.00[a]	2.20±1.10[a]	1.20±0.84[a]	1.20±1.64[a]	36.40±5.44[b]	41.00±14.64[b]	36.40±8.75[b]	20.80±4.61[b]

[a,b]Different superscripts in the same raw indicate a significant differences ($P<0.01$). SOV= Superovulation

continued to grow. This is because the PMSG mechanism works by recruiting small follicles and stimulating the growth of these follicles into large follicles (Supriatna 2018). As a result, large follicles were found to respond to superovulation. This large follicle enlarges twice bigger than its size before the superovulation treatment. Putro (2013) also reported that the addition of gonadotropin-releasing hormone (GnRH) resulted in the speed of development of ovulatory follicles, causing the small and medium follicles to continue to develop into large follicles. The picture of the comparison of the diameters of the follicles is clearly seen in Figure 1, which shows the number of follicles was directly proportional to the size of the ovaries which also grew after the superovulation treatment.

The number of large follicles during estrus (D14) differed between treatment groups, as shown in Table 2. The difference in natural estrus between Group A1 and Group A2 was not statistically significant (P>0.05) with an average value of 36.40±5.45 and 41.00±14.65, while the difference in PGF2α hormone-induced estrus between Group B1 and Group B2 was statistically significant (P<0.05) with a mean value of 36.40±8.75; 20.80±4.99. PMSG gave more varied responses in the PGF2α hormone-induced estrus group. Fauzi *et al*. (2017) stated that there were differences in the ability of each individual animal to secrete reproductive hormones optimally, thus affecting the PGF2α response in lysis of CL. Supriatna (2018) stated that PGF2α has luteolytic activity that causes a decrease in progesterone concentration so that the blockade of GnRH release can be neutralized indirectly by PGF2α luteolytic.

The increase in the number of large follicles continued to occur during estrus (after the superovulation treatment) on D14 vs. D21, both in the natural estrus group and PGF2α hormone-induced estrus group (see Table 2), with an average value of A1 (36.40±5.45 vs. 42.80±5.95), A2 (41.00±14.65 vs. 49.40±11.69), B1 (36.40±8.75 vs. 44.60±7.48), and B2 (20.80±4.99 vs. 49.20±7.85). Large follicles (dominant follicles) from all groups could not ovulate on D14, possibly due to the presence of CL at the time of superovulation which could not be lysed by prostaglandin injection on day 12 using estrumate (250 g cloprostenol/mL) which should be 500 g cloprostenol (Hatvani *et al*. 2013); thus, it is suspected that progesterone was still being produced. This progesterone hormone has a biopotential to inhibit the production and secretion of endogenous LH. Basal concentrations of endogenous LH are insufficient to induce an ovulatory response (Crowe *et al*. 2014). In addition, PMSG has a half-life span of 123 hours (about 5 days). Stimulation of superovulation occurs for 4 days to obtain mature follicles (dominant follicles). PMSG has FSH and LH biopotency with a FSH:LH ratio of around 60:40%. The 40% exogenous LH biopotential concentration of PMSG is still active on the 6th day of superovulation,

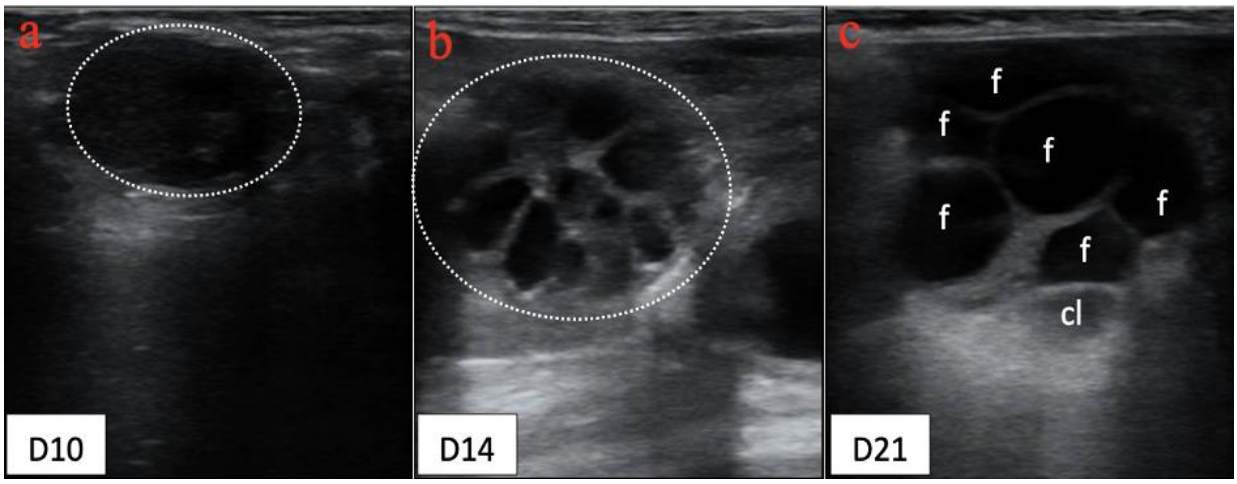

Figure 1. The development of ovarian follicles per day was observed using ultrasound imaging. F= Large follicle, CL= Corpus luteum, a= Picture of ovaries before PMSG induction, b= Picture of ovaries after PMSG induction, c= Picture of ovaries after SOV treatment

Table 2. Total follicles and corpus luteum after the administration of hCG 2000 IU during estrus PMSG treatment in superovulation

Donor groups	Treatment groups	Total follicles and CL after superovulation treatment		
		D14	D21	
		Large follicle	Large follicle	Corpus luteum
Natural estrus	A1 (+hCG)	36.40±5.45^{a}	42.80±5.95^{a}	1.20±0.71^{a}
	A2 (- hCG)	41.00±14.65^{a}	49.40±11.69^{a}	1.00±0.84^{a}
PGF2α hormone-induced estrus	B1 (+hCG)	36.40±8.75^{a}	44.60±7.48^{a}	1.60±1.14^{a}
	B2 (- hCG)	20.80±4.99^{b}	49.20±7.85^{a}	0.80±0.84^{a}

^{a,b}Differenet superscripts in the same column indicate a significant differences (P<0.05)

so it can suppress endogenous LH secretion from the anterior pituitary. The current LH concentration was the exogenous LH concentration which had already begun to decrease by half (it had decreased by 50% from the 40% PMSG exogenous LH concentration), and the existing LH concentration was insufficient to provide an ovulatory response from the dominant follicles resulting from PMSG superovulation (Supriatna 2018). As a result, no ovulation occurred on D21. On D21, the difference in the number of dominant follicles in the two treatment groups was not statistically significant (P>0.05). The mean values of A1; A2; B1; and B2 groups were 42.80±5.95; 49.40±11.69; 44.60±7.48; 49.20±7.85, respectively, as shown in Table 2. On D21, the number of large follicles was greater than on D14. This is because the large follicles (dominant follicles) formed at D14 did not ovulate; thus, follicular development continued. This happened because of the negative rebound effect of PMSG which had a long half-life (123 hours), causing follicle formation to continue to occur (Supriatna *et al.* 1998). Therefore, the administration of PMSG must be balanced with the administration of anti-PMSG to suppress the effects of PMSG. This is in line with the research results of Katagiri *et al*. (1991) in which PMSG biopotency was still detected through immunoassay examination on day 10 after injection, but PMSG levels decreased rapidly after anti-PMSG injection.

The addition of hCG after PMSG administration in this study did not show a statistically significant change (P>0.05) in the ovulation rate on D21 in the natural estrus groups (A1 and A2) and the PGF2α hormone-induced estrus groups (B1 and B2). However, it can be seen that the number of large follicles in each group injected with hCG was lower than the group without the addition of hCG (A1<A2 and B1<B2), but not significantly different (P<0.05). In addition, the groups given hCG induction continued to have an increase in the number of large follicles, just like the groups without the addition of hCG (see Table 2), with group mean values of A1 (36.40±5.45 vs. 42.80±5.95), A2 (41.00±14.65 vs. 49.40±11.69), B1 (36.40±8.75 vs. 44.60±7.48) and B2 (20.80±4.99 vs. 49.20 ±7.85).

The continued increase in the number of follicles on D21 may be due to the negative rebound effect caused by PMSG. This occurred because of the low response of hCG in increasing the rate of ovulation, which caused the biological activity of PMSG to continue. There was no endogenous LH surge because exogenous LH from PMSG could cause inhibition of LH secretion from the anterior pituitary, whereas the biopotency of exogenous LH from both PMSG and injected hCG was not sufficient to induce ovulation (Supriatna 2018). This indirectly affected the amount of CL produced (Cabrera *et al*. 2021). As a result, the amount of CL produced in the groups injected with hCG and without the addition of hCG did not increase significantly (P>0.05).

The number of ovulations was assessed by the total CL formed in both ovaries (Blitek *et al*. 2016). Hazano *et al*. (2020) in their research stated that administering hCG to crossbreeding heifers on day 5 post-ovulation can induce the ovulation of the dominant follicle and the formation of accessory CL and reduce plasma estrogen concentrations. However, the effect of hCG on a cow's ovarian function is also influenced by the location of the CL and the dominant follicle. In addition, Sarsaifi *et al*. (2013) concluded that hCG dose is the most prominent factor in achieving the rate of oocyte recovery and maturation. This is in line with the research results of Cabrera *et al*. (2021) that compared the effects of hCG administration at four different dose levels, namely 1000 IU, 2000 IU, 2500 IU and 3300 IU, which produced a better ovulatory response in cattle at doses of 2500 IU and 3300 IU. It is possible that in this study, the induced dose of hCG was still insufficient to stimulate ovulation.

In this study, it can be stated that PMSG gave good responses to the group of donor cows with natural estrus and the group with PGF2α hormone-induced estrus. After the administration of hCG, the rate of follicular growth in both treatment groups continued to increase. This may be due to the high levels of progesterone caused by the insufficient dose of cloprostenol injection (Hatvani *et al*. 2013), thereby inhibiting the release of endogenous LH. In contrast, the biopotency of exogenous LH produced from PMSG after 5 or 6 days after the superovulation PMSG injection was only half because it had exceeded the PMSG half-life time limit of 123 hours (5 days) and was less than its basal concentration to be able to cause an ovulatory response from mature follicles resulted from superovulation. It caused abnormal follicular development with large and persistent follicles. Jodiansyah *et al*. (2013) stated that if superovulation is carried out when the follicles have low sensitivity to hormones, fewer follicles will be stimulated to develop. In addition to hCG, the PGF2α hormone also plays a role in increasing the responsiveness of the pituitary gland to produce GnRH, which increases the release of LH and induces ovulation (Pfeifer *et al*. 2014). At the biphase stage, female cows have mature follicles, active CL, and high concentrations of the hormones estrogen and progesterone in the blood. If superovulation results in a large number of mature follicles but the CL has not been completely lysed due to insufficient injection of prostaglandins and the half-life of prostaglandins being only 25 seconds, the response can be less effective and the injection of prostaglandins will need to be repeated. The half-life of PGF2α is so short that optimal doses are needed to prolong its action in order to reach the receptor target (Pfeifer *et al*. 2018).

CONCLUSION

Superovulation using PMSG gave the same results to the donor cows with natural estrus and the donor cows with PGF2α induced estrus. The hCG hormone with a dose of 2000 IU injected i.m. was also not able

to increase the ovulation rate of Aceh cattle superovulated with PMSG 2500 IU i.m.

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