

ASSESSMENT OF COMBINATION TREATMENT OF HORMONAL AND ARTIFICIAL INSEMINATION (AI) FOR TWINNING CALF BIRTH

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ABSTRACT

Problem of beef cattle farming are low reproduction performance with long calving interval, low number calf/year, high service per conception and low cow fertility. In term of accelerating improvement of beef cattle population, it needs proper technology to increase percentage of twinning calf birth (twinning birth rate) through superfetation, superovulation and zygote cleavage. The study was aimed to find out the effective and efficient proper technology by combination treatment of hormonal and artificial insemination (AI) to increase twinning birth calf. The study was executed in Sumberkembar, Binangun – Blitar between Sept 2011 – March 2012 using 7 combination treatments between hormonal and AI frequency for 21 cows with 2 replications and 3 heads cow each: control (without treatment under traditional farm management) with 1 time AI (12 hours after oestrus signed); 2) Treatment A: FSH 4 cc, intra muscular with 1 times AI (12 hours after oestrus signed); 3) Treatment B: FSH 4 cc, intra muscular injection with 2 times AI (12 hours after and 2nd oestrus signed); 4) Treatment C: FSH 4 cc, intra muscular injection with 3 times AI (12 hours after, 2nd and 3rd oestrus signed) 5) Treatment D : PMSG 1000 IU injection with 1 time AI (12 hours after oestrus signed), 6) Treatment E: PMSG 1000 IU injection with 2 times AI (12 hours after and 2nd oestrus signed); and treatment F: PMSG 1000 IU injection with 3 times AI (12 hours after, 2nd and 3rd oestrus signed). The completely randomized design with factorial pattern (2 hormonal administration and 3 AI frequencies) were used with 7 different treatments and 3 replications. The collected data were tabulated and analyzed using Anova and Duncan test using linier model procedure (SAS, 1996). Result showed a different result between control and hormonal treatment (PMSG dan FSH), but no different result was found among hormon. The AI frequency have significant effect on blood progesteron concentration of the cow. The higher AI frequency, the higher probability to produce twinning birth with higher blood progesteron concentration. The AI 1, 2 and 3 times have blood progesteron concentration: 3.28; 8.3 and 15.44 ng/ml blood, but no significant different was found in their interaction of hormonal treatment and AI frequency.

Key words: Hormonal, Artificial Insemination and Twinning Calf

INTRODUCTION

Background

Macroeconomic objective of agricultural development in 2010 is increasing 4.6% gross domestic product, while the target of major food commodities could reach 399,500 ton of beef, primarily to support the program of national beef self-sufficiency in 2014, through efforts to minimize target gap between national demand and supply of beef decreased became 9.8%. Although it was reported, that the realization of cows calving from artificial insemination results in East Java from 2003 – 2007 there was an increase of about 18.9% (Anonymous, 2007), but the demand of

national beef and cattle was fulfilled through importation.

East Java as a national supplier of 30% beef, in 2009 produced 3,499,200 heads of beef cattle, or 85,173,356 kg of beef and spread in East Java district (East Java Livestock Office 2009) or increased 3.37% compared to 2008. To achieve beef self-sufficiency in East Java province in 2014 East Java Livestock Office launched a program of "Diamond Cows", which targets are the achievement of the birth of five (5) million cows in five (5) years of the effective activities of Artificial Insemination (AI) with a target average growth of beef cattle 6.18% of births so that the target period of 2009 until 2014 total of 5,271,296 heads will be achieved. In order to accelerate the increase

of cattle population it needs alternative effort by enhancing the percentage/frequency of twins (twinning birth rate) in the beef cattle.

Twinning can occur because of superfetation, superovulation and zygote cleavage into two new individual immediately after fertilization. Superfetation twins are not scientifically categorized into the twin events. Superfetation twins occur because cows are already mating and pregnant after 7 – 14 days showed estrus signs then young and pregnant cows are mated again and had subsequent pregnancy is called superfetation twin. Thus the age of the embryo in the uterus is not the same so that it will have calving time such a time span of mating. In general, the frequency of young pregnant cows return estrus is 1 – 2% of total pregnancy.

A good mating management is also important to improve the efficiency of reproduction, including heredity improvement. One way to improve livestock management is by artificial insemination (AI) that the efficacy already has been enjoyed for decades. AI techniques can enhance genetic improvement quickly, prevention livestock infertility, preventing spread of diseases, thus increasing beef production is not impossible (Anonymous, 2009).

Hardini *et al.* (2010) suggested that there was influence of PMSG (pregnant mare serum gonadotropin) injections up to 30% chance of twin pregnancy, observed from the results of the CL on days 19 and 30 after IB. Chance of producing twins pregnancy needed support of feeding conditions such as good quality and quantity feed.

Problems

One of the problems in beef cattle business is a relatively low reproductive performance. Beef cattle management conditions at small farmer are still traditionally that caused low productivity and reproductivity of beef cattle. Low performance reproduction are generally reflected by the length of calving interval, low calving per year, high service per conception (S/C) and low fertility. From the various reports in East Java, it is mentioned that the existence of large variation in the level of livestock productivity, in additional cases

found that reproductive failure. It is expected that AI application and appropriate hormone dose and also mating time is one way to improve reproduction performance of beef cattle cow.

The purpose of this study was to compare calves twin technology through application of FSH and PMSG and also AI to support twinning pregnancy.

MATERIAL AND METHODS

Assessment was conducted for one year, starting in August 2011 until July 2012. The location was in Sumber Kembar village, Binangun sub-District, Blitar District.

Assessment plan

The assessment was done using complete randomized design with seven treatments, each treatment used 3 cows as replicates.

Materials

This research used 21 heads cows, selected by:

- Body Condition Score (BCS): 5 – 7 (Nicholson and Butterworth, 1986)
- Normal body condition especially reproduction tract checked by rectal palpation
- Not have a history of illness or reproductive disorders.
- Ever calving before at least once and physiological status.

Implementation of a detailed technical assessment activities presented in Table 1. Each farmer was given a disinfectant at the beginning of cooperative activities to clean and maintain the pen, cattle and the environment to make sure not affect the outcome of the assessment activities. Administration of oral de-worming was done at the beginning of the assessment to treat and prevent cows from worms that will affect the physiological and metabolic condition of livestock. Feeding for all assessment materials was 3.5 kg of concentrate per head per day for 2 months to improve cows conditions and preparation of

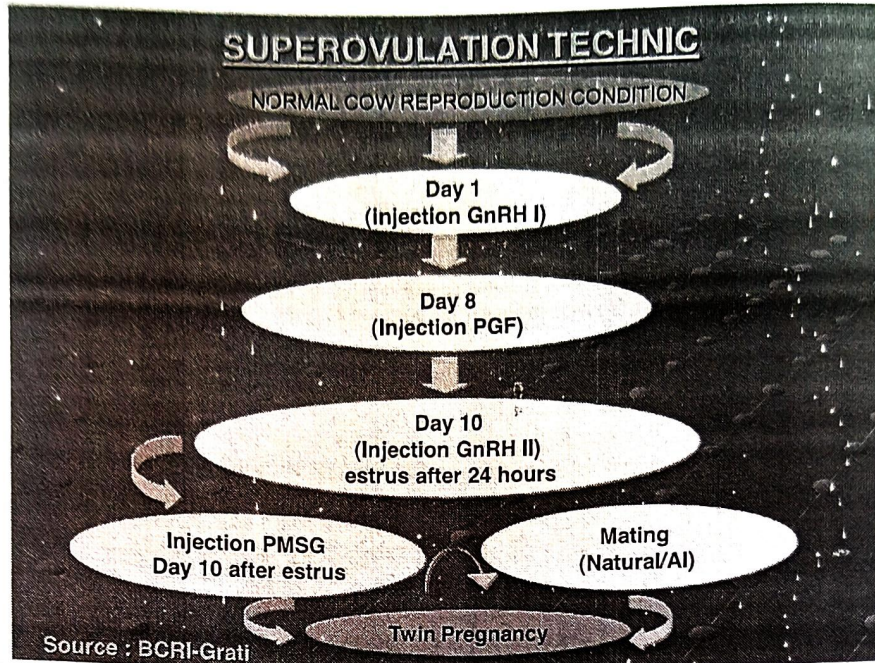


Figure 1. PMSG Treatment for super ovulation

Tabel 1. Assessment treatment combined hormone dan AI for beef cattle cow

Item	Control C	Treatment					
		A	B	C	D	E	F
Hormone	-	FSH, 4 cc, IM	FSH, 4 cc, IM	FSH, 4 cc, IM	PMSG, 1000 IU, IM	PMSG, 1000 IU, IM	PMSG, 1000 IU, IM
AI	Once	Once	Twice	Three times	Once	Twice	Three times

cow body condition before pregnancy, the nutritional content ranging from: DM (dry matter) = 87 – 88%; CP (crude protein) = 12 – 13%, TDN (total digestible nutrients) = 62 – 65%. The provision of mineral nutrients to add value to cattle ranchers also performed. Situmorang (2010) recommendations for the hormonal treatment (FSH) method was described according to Table 2.

Observations/processing and data analysis

- CL (corpus luteum) observation is performed at least 19 days after AI by rectal palpation.
- Blood progesterone levels (ng/ml) using Enzyme Immunoassay Test Kit Catalog Number: 4S00121, done shortly before the

injection of the hormone (H0), day 19 and day 45 after AI.

Tabel 2. FSH treatment (Situmorang, 2010)

Detail activities	Time
PGF 2.5 cc	Day 1 st
Pengamatan estrus	
FSH 1 st	Day 14 th
FSH 2 nd	Day 15 th
FSH 3 th	Day 16 th
FSH 4 th	Day 17 th

Processing and data analysis

The results of assessment data were tabulated and analyzed of variance using the

general linear model procedure (SAS, 1996). Significant differences between treatments were tested with the smallest difference test and comparison test for each of the quantitative and qualitative parameters.

RESULTS AND DISCUSSION

The identity of the research material

The cows that were used as research material were listed in Table 3. Cows used in this assessment was local beef cattle PO, currently it is difficult to find PO cows because of the population is rare. It is important to maintain the existence of the local cattle otherwise it will become extinct in the next period. Small farmer prefer had cross breed cow than local cow because of cross breed performance such as bigger size, and stronger and also high value for sale. But it must consider that good performance of crossbreed

cows need good input as well, especially feed. Feed quality with an adequate amount is necessary to get optimal livestock productivity. The Excellent trait of local cattle is high adaptability with poor feeding (quality and quantity) and the adaptation to heat stress that did not had by cross breed cattle.

Cows BCS (body condition score) are generally 5, this indicated that cows received less feed and farmers gave less attention to cattle feeding, both the quality and quantity. Type of feed that were given such as hay and some farmer gave few grass, because the condition of dry land farmers have trouble getting fresh forage. Concentrate feeding for 2 months is expected to improve cow body condition, especially for preparing pregnancy and calving. Evident after one month of concentrate feed given the condition of cows are better and fatter than the initial conditions before the study was conducted (generally up 1 point).

Table 3. Cattle identity that used in this assessment

Farmer name	Breed	BCS	Parity	Girth (cm)
Sumarno	PO	5	4	155
Sutikno	PO	5	2	155
Widodo	PO	5.5	5	164
Marjiyanto	PO	5	3	152
Mulyadi	PO	5	2	153
Kasdi	PO	5.5	4	165
Sudar	PO	6	4	168
Saniran	PO	5.5	3	165
Supriyanto	PO	7.5	2	185
Sauji	PO	5	5	160
Kateman	PO	6	3	168
Somo S	PO	5	3	163
Redi	PO	6	4	175
Suhar	PO	5	2	160
Juri	PO	6	3	172
Sukondo	PO	5	4	158
Samin	PO	5	5	155
Sutrisno	PO	5	3	170
Bonimin	PO	5	1	163
Bonimin	PO	5	1	167
Bonimin	PO	5	1	164

Corpus luteum (CL) and heart beat detection result

The results in percentage of CL examination showed that most cows (84.62%) were not detected and some cows that kept by Saniran and Kasdi were detected (15.38%). This is because the investigation is still too early, 10 – 30 days after the AI and therefore can not demonstrated twin pregnancy status. Used doppler device for pregnancy diagnosis at age > 2.5 months.

PMSG hormone application method is essentially similar to the method of FSH application but PMSG only need one shot on day 10 after the cows showed estrus signs or on day 14 after injection of PGF (prostaglandin). Injection of GnRH to improve the condition of the ovaries in order to optimize the function of ovaries, while the prostaglandin is one of the preparations that are often used for estrus synchronization program that could regress corpus luteum (Milvae *et al.*, 1996). Previous research stated that PGF injection for estrus synchronization program performed twice injection with interval 11 – 12 days and estrus will appear between days 2 – 7 and the highest concentration on day 3 after second injection (Mac Millan, 1983). Application of PMSG or FSH aims to create a superovulation so that each time the cow estrus can more than one egg and sperm from the male can inseminate more than one egg.

Ovulation is releasing ovum as a result of rupture of the follicle that has been matured. The time needed by the whole process of ovulation depends on the location of the egg in the follicle. Short time of ovulation when the egg will be on the basis of the follicle and will be long if the egg is close to the stigma that protrude from the ovary. As the follicles grow because of the influence of anterior pituitary hormone FSH from the follicle cells capable of producing estrogen and progesterone. Both of these hormones in small doses will cause the release of the hormone LH. LH plays an important role in ovulation bluffing. Rupture of follicles occurred between the pressure of the follicles that grow on the stigma and rips pale because this region lacks the blood.

Stimuli on both the outer cervix during copulation or artificial breeding will be forwarded by the nerves to the central nervous

system to be received by the hypothalamus. It would then realising hormone secreted LH and LH levels in the blood will increase, resulting in ovulation. The remnants of the follicle that has ovulated will form a variety of fabrics, namely: 1) corpus haemoragikum, after ovulation will follow the provision of more blood to the remnants of the follicle which then would happen hypertrophy and hyperplasia of the fabric to form a spherical object protrude from the ovary, chewy and colored red; 2) the corpus luteum as a result of the process of corpus haemoragikum luteinizing hormone by the effect of LTH, there was further growth of these cells. New weave will change color to yellow and produce progesterone that long will be high at the peak of the cycle heat and 3) albikansia corpus, namely the cessation of activity in the corpus luteum produces progesterone will cause the degeneration of the cells because they do not get the shape of the blood supply becomes very small and pale colored (Mac Millan, 1983).

Ovulation in cattle occurs in 10 – 12 hours after estrus ended, a disturbance at the time of ovulation can lead to the absence of fertilization and embryonic development or disturbance. Ovulation disorders may occur due to deficiency or imbalance of the endocrine and other factors.

The analysis of blood progesterone levels

Bovine blood progesterone concentration showed at Table 4.

Application of different hormones showed highly significant difference ($P < 0.01$) for blood progesterone concentration compared to the cow without the hormone/control. Hormone concentration of progesterone was higher for cows that received FSH or PMSG compared to control, this meant that the possibility of pregnant cows was higher in cows given the hormone. There was positive correlation of blood levels of the hormone progesterone with the pregnancy condition of the cow, the older age of pregnancy the higher the blood levels of the hormone progesterone cow, this is because the hormone progesterone is protective to the fetus in uterus.

AI intensity was also significant difference between AI once, twice and three times ($P <$

Table 4. Concentration of progesteron hormone in blood (ng/ml)

Cows	Hormone			Average
	Control	PMSG	FSH	
AI once	2.00	3.95	3.90	3.28 ^a
AI twice	-	8.95	7.65	8.30 ^b
AI three times	-	15.50	15.38	15.44 ^c
Average	2.00 ^a	9.47 ^b	8.98 ^b	

0.01). Cow that received AI three times showed the highest progesterone levels, followed by AI twice and once. Correlation between AI intensity with progesterone hormone levels were influenced by the success of pregnancy because of AI and fetus conceived in cows. AI application for cow with estrus more than one time can increase the chances of twins superfestation, where the fetus can be different age and phenotype. Another possibility occurs in the cow who had AI more than once are abnormalities in the reproductive organs, such example diseases that disrupt the reproductive tract, reproductive hygiene before or during delivery may also be genetic.

Twin superfestation are rare, however, influence the intensity of the AI and the hormone can increase possibility of twinning. This is evident in the results of the assessment there were abort cows (age pregnancy 5 months) with 3 fetuses (Figure 2).

Abortion in cattle can occur because: (1) the number of fetuses in the uterus more than one so that the space and opportunity to get enough nutrients is very small; (2) Pregnant cow received less feed; (3) Accidents, example falls, slips, etc.

Super ovulation program costs

Super ovulation using hormones PMSG and FSH was presented in Table 5. Table 5 shows that the cost is quite expensive using hormone application either PMSG or FSH, it cost IDR 775,000 and IDR718 750, compare to farmer method (control), IDR 50,000 assuming for once AI. It cost higher application fee if the AI performed more than once, efficient and economic analysis can not be calculated because the results of twinning birth have not complete.



Figure 2. Twin fetuses death because abortion (5 months)

Tabel 5. Superovulation program cost using PMSG dan FSH (in IDR)

Cost	Control	PMSG			FSH		
		AI 1 ×	AI 2 ×	AI 3 ×	AI 1 ×	AI 2 ×	AI 3 ×
IB	50,000	50,000	100,000	150,000	50,000	100,000	150,000
PMSG							
GnRh 5 ml	-	206,250	206,250	206,250	-	-	-
PGF 2.5 ml	-	68,750	68,750	68,750	-	-	-
PMSG, 1000 IU	-	450,000	450,000	450,000	-	-	-
FSH							
PGF, 2.5 cc	-	-	-	-	68,750	68,750	68,750
FSH 4 cc	-	-	-	-	600,000	600,000	600,000
Total	50,000	775,000	825,000	875,000	718,750	768,750	818,750

CONCLUSIONS

The conclusions of this activity are:

1. Results showed CL was 15.38% while the rest have not shown yet.
2. Application of different hormones highly significant affected ($P < 0.01$) cow blood progesterone levels compared to cow without the hormone/control. Hormone levels of progesterone cow were higher either received FSH or PMSG than controls.
3. AI intensity was also significantly differed between AI 1x, 2x and 3x ($P < 0.01$). Cows received AI three times showed highest progesterone levels, followed by cow received AI 2x and 1x.
4. Hormone application fee with once AI either using PMSG or FSH cost IDR 775,000, and IDR 718,750 respectively. Farmer's method (control) only cost IDR 50,000.

REFERENCES

- Anonymous. 2007. Data populasi dan sebaran ternak sapi potong di Jawa Timur 2007. www.disnak-jatim.go.id (28th January, 2009).
- Anonymous. 2009. Sapi Kembar tingkatkan produksi daging Sulsel. <http://www.tribun-timur.com> (28th January, 2009).
- Dinas Peternakan Provinsi Jawa Timur. 2009. Laporan Tahunan Provinsi Jawa Timur. www.disnak-jatim.go.id (June 2010).
- Hardini, D, M.A. Yusran, A.H. Ariyanto, M.A. Mualif, Daldiri dan S. Koessusianna. 2010. Pengkajian Teknologi Reproduksi (Stimulasi hormon, Jarak Beranak 12 Bulan) dan Pakan Lokal (Kelahiran Kembar $\geq 50\%$) pada Sapi Berpotensi Beranak Kembar di Jawa Timur. Laporan akhir kegiatan kerjasama Badan Litbang Pertanian dengan Menteri Negara Ristek dan Teknologi Republik Indonesia.
- MacMillan, K.L. 1983. Prostaglandine response in dairy herd breeding programs. *J. Vet.* 31: 110 – 113.
- Nicholson, M.J. and M.N. Butterwoth. 1986. A Guide to Condition scoring of Zebu Cattle. International Livestock Centre for Africa, Addis Ababa. 26 p.