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APPLICATION OF ESTRUS SYNCHRONIZATION USING PGF2α AND OVULATION SYNCHRONIZATION USING hCG FOR ARTIFICIAL INSEMINATION OPTIMIZATION ON ONGOLE BREED CATTLE

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ABSTRACT

This study aimed to determine the pregnancy percentage of Ongole breed (PO) cattle by estrus synchronization and ovulation synchronization. This study used 22 cattle that were divided into three groups namely estrus synchronized cows (K1, n= 5); ovulation synchronized heifers using ovsynch (K2, n= 6); and ovulation synchronized cows using ovsynch (K3, n= 11). Parameters measured were diameter of corpus luteum (CL) in estrus synchronization, follicular diameter upon synchronization and artificial insemination (AI), and percentage of pregnant cattle. Data obtained were statistically analyzed using analysis of variance. Results showed no significant differences (P>0.05) of CL diameter at the time of estrus synchronization in all groups of cattle with an average of 16.63±3.79 mm. The CL diameter at the time of estrus synchronization was not significantly different among groups, with an average of 8.80±2.07 mm. Diameter of follicles during ovulation synchronization was also not significantly different among groups. The average diameter of follicles was 9.01±2.05 mm. Diameter of follicles at the time of estrus and ovulation synchronization was not significantly different among groups with an average diameter of follicles of 10.94±2.10 mm. The pregnancy percentage of K1, K2, and K3 were 60%, 16%, and 36%, respectively. There was no correlation between the diameters of follicles during estrus with the pregnancy percentage. Estrus synchronization produced higher pregnancy rate than ovulation synchronization in cows or heifers.

Key words: estrus synchronization, ovulation synchronization, PO cattle, pregnancy percentage

ABSTRAK

Penelitian ini bertujuan mengetahui persentase kebuntingan hasil sinkronisasi estrus dan sinkronisasi ovulasi pada sapi Peranakan Ongole (PO). Dalam penelitian ini digunakan 22 ekor sapi yaitu induk sinkronisasi estrus (K1, n= 5); dara ovsynch (K2, n= 6); induk ovsynch (K3, n=11). Parameter yang diamati adalah diameter korpus luteum (CL) saat sinkronisasi estrus, diamater folikel pada saat sinkronisasi dan inseminasi buatan (IB) serta persentase sapi bunting. Data yang diperoleh dianalisis secara statistik menggunakan analisis varians. Hasil penelitian menunjukkan tidak ada perbedaan nyata (P>0,05) terhadap diameter CL pada saat sinkronisasi estrus pada semua kelompok sapi dengan rata-rata 16,63±3,79 mm. Diameter CL pada saat estrus tidak berbeda nyata antar kelompok dengan rata-rata 8,80±2,07 mm. Diameter folikel pada saat sinkronisasi ovulasi tidak berbeda nyata antar kelompok dengan rata-rata diameter folikel adalah 9,01±2,05 mm. Diameter folikel pada saat estrus dan sinkronisasi ovulasi tidak berbeda nyata antar kelompok dengan rata-rata diameter folikel 10,94±2,10 mm. Persentase kebuntingan pada K1, K2, dan K3 berturut-turut adalah 60%, 16%, dan 36%. Tidak terdapat korelasi antara diameter folikel pada saat estrus dengan persentase kebuntingan. Sinkronisasi estrus pada sapi induk menghasilkan kebuntingan yang lebih tinggi dibandingkan sinkronisasi ovulasi pada sapi PO induk maupun dara.

Kata kunci: sinkronisasi estrus, sinkronisasi ovulasi, sapi PO, persentase kebuntingan

INTRODUCTION

Ongole breed (PO) cattle, one of the local hybrid beef cattle, are a source of meat in Indonesia which population can be developed. The population of PO cattle is expected to meet 90-95% of national meat needs (Ditjennak, 2014). Development and optimization of PO cattle production can be realized through artificial insemination (AI).

Estrus synchronization can overcome the problems in AI implementation and resulted in higher conception rate (Larson *et al*. 2006). One way to apply estrus synchronization protocol is by using PGF2α hormones that are luteolytic for corpus luteum (CL). Percentage of estrus cattle injected by PGF2α on day 7 of the estrous cycle amounted to 87.8% (Laverdibre *et al.* 1995). Administration of two doses of PGF2α on day-5 induces 95-100% CL regression and when the cattle were inseminated 72 hours after the last PGF2α, the pregnancy percentage can reach 62.1% (Peterson *et al.* 2011).

The use of human chorionic gonadotropin (hCG) in ovulation synchronization protocol (ovsynch) in cattle may allegedly cause increase of conception rates. Ovsynch method in dairy cows with 1,500 IU hCG resulted in conception rate of 37.6% (Keskin *et al.*, 2010). Administration of 1,000 IU hCG in cows before and after AI resulted in pregnancy rate of 49.1% (Marquezini *et al.*, 2011). This study was conducted to determine the effectiveness of using PGF2α estrus synchronization and ovulation synchronization with hCG followed by AI to see the percentage of pregnancy in PO cattle.

MATERIALS AND METHODS

This study used 16 cows aged 3.5 years, had at least one calf, had body weight of 280 kg and 6 heifers aged 2 years with a body weight of 250 kg, and had reached adulthood marked by CL presence. All cows and heifers showed no reproductive tract infections observed by ultrasonography (USG). Cattle were given feed of tofu, wheat, cassava, yams, and grass.

Reproductive Status Determination

PO cattle reproduction status were observed with ultrasonography machine (SonoDrop Vet A6, China) by placing a probe (5 MHz linear probe, China) through the rectum and the reproductive organs image was recorded with printer (SONY UP-895 MD, Japan). Parameters measured were CL and follicle diameters that were measured by an internal caliper on an ultrasound device defined by the distance between the two points with the longest axis millimeters (mm). CL diameters ≥15 mm was set as luteal phase, then estrus synchronization were performed.

Estrus and Ovulation Synchronization

Cattle were divided into three treatment groups. Group 1 (K1) consists of 5 PO cows which received estrus synchronization using 10 mg of PGF2α (Noroprost®, Dinoprost 5 mg/mL, Intervet, Cambridge) intravulva submucosal (ivsm) in the luteal phase. Group 2 (K2) consist of 6 heifers which received estrus synchronization with PGF2α in the luteal phase and ovulation synchronization with 1,500 IU of hCG (Chorulon®, Intervet Chambridge) given intravenously (iv) at the time of estrus. Group 3 (K3) consist of 11 cows which received ovulation synchronization as Group K2 (Figure 1). Estrus occurrences were observed in the morning and afternoon by observing estrus symptoms such as swollen vulva, flushing, produced mucus, and restless. Artificial insemination was performed on the first day of estrus and on the second day if the estrus symptoms still present. AI was carried out by the same inseminator using frozen semen of Simmental cattle produced by Lembang Artificial Insemination Centers. Pregnancy examinations performed by ultrasound at day 30 after the last AI. Pregnancy was marked by the emergence of anechoic fluid in the uterine lumen and the presence of a fetus as a hypoechoic structure in the amniotic fluid and the presence of the cotyledons on the uterine wall.

Data Analysis

CL and follicles diameter before PGF2α injection and before AI in every group and among treatment groups as well as pregnancy percentage were analyzed using analysis of variance (ANOVA) (Steel and Torrie, 1991).

RESULTS AND DISCUSSION

The results showed that the diameter of the CL on K1, K2, and K3 were not significantly different (P>0.05) before the injection of PGF2α and before AI performed (Table 1). PGF2α injection occurred after CL regression process characterized by a decrease in the diameter of the CL, marking the beginning of follicular phase and causing estrus. This was consistent with the research results from Melia (2010) in PO cattle that showed a decrease in the size of CL from day 1 after injection (1.53±0.12 cm) up to day 3 (0.93±0.12 cm) when estrus occurred, CL was not visible, indicating that the CL was completely lysed.

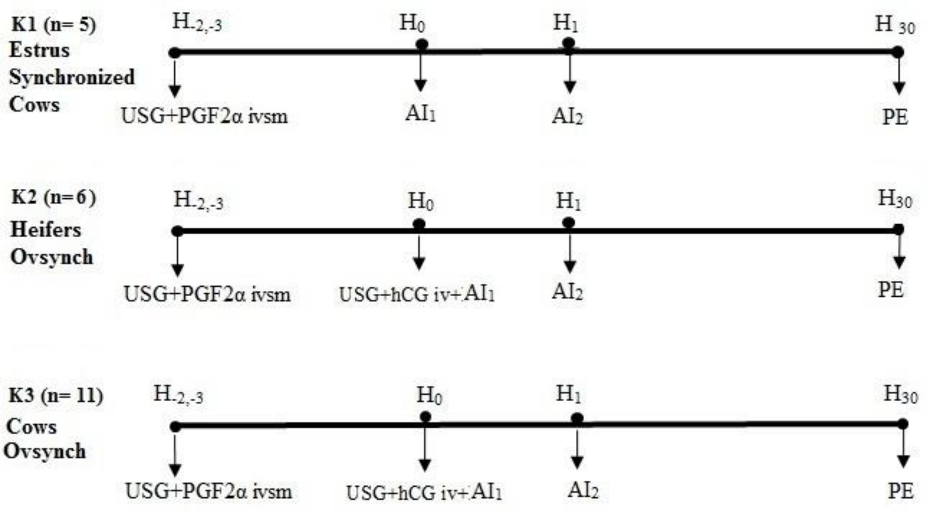

Figure 1. Research procedure. $H_{-2,-3}$= Luteal phase of synchronized estrus, H_0= Estrus= AI_1, H_1= AI_2, H_{30}= Pregnancy examination, USG was used to determine the reproductive status, PGF2α (ivsm)= Intravulva submucosa PGF2α, AI= Artificial insemination, hCG iv= Intravena hCG

Table 1. The average diameter of the CL and follicles during estrus synchronization (before PGF2α) and after ovulation synchronization (before AI) and the pregnancy percentage in PO cattle

Group (K)	Before $PGF_2α$		Before AI		Pregnancy percentage
	CL (mm)	Follicles (mm)	CL (mm)	Follicles (mm)	
1 (n= 5)	18.12±5.30	8.30±2.13	9.34±1.96	11.55±2.22	60%
2 (n= 6)	17.60±3.06	9.30±2.53	9.73±3.08	10.38±1.86	16%
3 (n= 11)	14.18±3.02	9.45±1.49	7.33±1.18	10.90±2.22	36%
Mean	16.63±3.79	9.01±2.05	8.80±2.07	10.94±2.10	

PGF2α hormone responses on cattle with a regular cycle in the luteal phase would effectively stimulated estrus. PGF2α action in luteolysis through counter-current mechanism caused a decrease in progesterone levels. As a result, there was progesterone negative feedback on the hypothalamus which increases gonadotropin hormone production which later stimulated anterior pituitary to secrete the follicle stimulating hormone (FSH) and luteinizing hormone (LH). Follicle stimulating hormone stimulated the development of follicles which would increase the production and secretion of estrogen. High estrogen concentrations induced estrus and provide positive feedback on the hypothalamus, causing a surge in LH that stimulated ovulation of the dominant follicle (Senger, 2005).

In line with the research conducted by Stötzel *et al*. (2012), the administration of PGF2α at mid-luteal phase caused luteolysis in a few hours which eventually would cause a decrease in the concentration of progesterone, estrogen increase, LH peak, and ovulation, then when AI was performed at 72 hours after administration of PGF2α, it resulted in pregnancy rate reaching 62.1%. The average decreased in the diameter of the CL and the increased in follicle diameter before estrus synchronization (before PGF2α) and after synchronization of ovulation (before AI) and the percentage of pregnancy in PO cattle were presented in Table 1.

hCG was used in estrus synchronization in cattle to induce ovulation simultaneously, modify follicular dynamics by increased the frequency of follicular waves up to three cycles follicular waves (De Rensis *et al*., 2010). The injection of hCG on day 7 of cattle cycle would induce the formation of CL and CL accessories that could increase the concentration of progesterone (3.7±0.3 ng/mL). The high concentration of progesterone could reduce the risk of early embryonic death (Marquezini *et al*., 2011).

The results showed that there was no significant difference in diameter of follicles before injection of PGF2α and AI among K1, K2, K3, and among K1, K2, K3 ($P>0.05$), which proved by the same follicle diameter, indicated that all cattle in K1, K2, and K3 were in the follicular phase. However, numerically there were increased trend of the follicle diameter in K1 (8.30±2.13 mm vs. 11.55±2.22 mm), K2 (9.30±2.53 mm vs 10.38±1.86 mm), and K3 (9.45±1.49 mm vs. 10.90±2.22 mm). This study also showed that there was no positive relationship between the diameter of the follicle and pregnancy rate in cows and heifers after estrus and ovulation synchronization. These results were in line with Gimenes *et al*. (2007) that the follicles with the size of 8.5-10 mm and ≥10 mm would provided ovulation response of 80% and 90%, respectively, while pregnancy rate did not differ significantly.

The K1 group, before AI, had larger diameter follicles and higher pregnancy rate (11.55±2.22 mm and 60%) than K2 (10.38±1.86 mm and 16%) and K3 (0.90±2.22 mm and 36%). The K1 that was injected with hCG revealed higher pregnancy percentage than the group that was not injected with hCG. Based on results, administration of hCG did not affect the increased of pregnancy rate in inseminated cattle. This was in line with the results from Marquezini *et al*. (2011), that stated that the administration of hCG in cattle resulted in pregnancy rate of 38.6% compared to 56.1% in control group which was not given hCG. Ovsynch method with administration of 3,000 IU of hCG to cattle inseminated once resulted in pregnancy rate of 36.7% (De Rensis *et al*., 2010).

According to Mussard *et al*. (2007), hCG administration could cause premature ovulation of small diameter follicles so that the ovulated follicles had low fertility and a decrease in luteal function, so the relatively low progesterone concentrations would be associated with high mortality of embryos. In addition, hCG acted directly on the ovary, not through the hypothalamus so that it did not induced gonadotropin-releasing hormone (Filicori *et al*., 2005) and the secretion of FSH or LH, where LH was important for oocyte maturation and ovulation (Shimada *et al*., 2003). hCG administration did not result in dominant follicle maturation due to insufficient LH surge thus cause low pregnancy rate (Yavas and Wallon, 2000).

Signs of 30 days gestation age after the last AI marked by the emergence of anechoic fluid in the uterine lumen and the presence of a fetus as a hypoechoic structure in the amniotic fluid and the presence of the cotyledons on the uterine wall. The viability of the developing fetus could be ensured when the beating fetal heart was visualized as pulsating hypo echoic structure (Curran *et al*., 1986). Ultrasound result was presented in Figure 2.

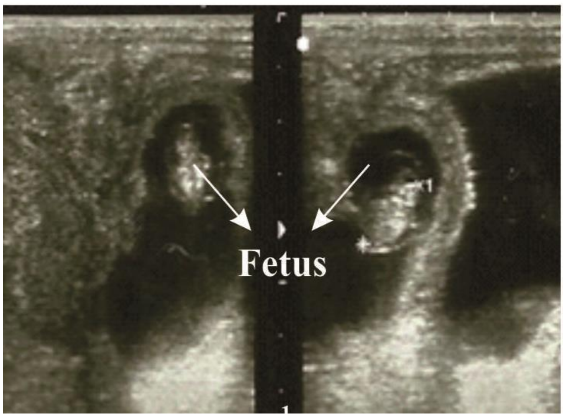

Figure 2. An ultrasound of 30 days old fetus

CONCLUSION

Estrus synchronization in cattle produced higher pregnancy than ovulation synchronization in cows and heifers.

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