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STEROID LEVEL AND PREGNANCY RATE OF ACEH COWS IN RESPONSE TO OVULATION INDUCTION USING PRESYNCH-OVSYNCH METHOD

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ABSTRACT

This study aimed to observe the steroid level and pregnancy rate in Aceh cows in response to ovulation induction using presynch-ovsynch method. Ten of two months post-partum non-pregnant and clinically healthy Aceh cows were divided into two groups of five cows each. First group (K1) was treated with presynch-ovsynch method. Second group (K2) was estrus synchronized using 5 mL of PGF2α, intramuscularly, administered twice with a 12-day interval. Both K1 and K2 were inseminated with frozen fertile semen 48-hours after the last treatment. The estrous observation was done after the last injection. Blood samples were collected immediately after the insemination for estradiol level quantification and on day-7 post-insemination for progesterone level measurement. The steroid level measurement was carried out using enzyme-linked immunosorbent assay (ELISA) technique. Pregnancy test was established on day-90 post insemination by rectal palpation technique. All of the cows showed symptom of estrous after the treatment. Estradiol and progesterone level of K1 vs K2 each were 294.98±110.48 vs 392.76±11.6 pg/mL (P>0.05) and 23.85±15.14 vs 12.69±5.64 ng/mL (P>0.05). Pregnancy rate of K1 vs K2 each were 60.0% vs 0.0%. Thus, presynch-ovsynch improved pregnancy rate but conversely did not elevate the estradiol level in Aceh cows.

Key words: Aceh cows, pregnancy rates, presynch-ovsynch, steroid level

ABSTRAK

Penelitian ini bertujuan mengetahui peningkatan level steroid dan persentase kebuntingan sapi aceh terhadap induksi ovulasi dengan metode presynch-ovsynch. Dalam penelitian ini digunakan sepuluh ekor sapi aceh betina dengan status tidak bunting, minimal dua bulan pascapartus, sudah pernah beranak, dan sehat secara klinis. Sapi dibagi atas dua kelompok, yang masing-masing terdiri atas lima ekor sapi. Kelompok pertama (K1) disinkronisasi berahi dengan metode presynch-ovsynch. Pada kelompok kedua (K2), disinkronisasi berahi menggunakan 5 ml PGF2α secara intramuskulus dengan pola penyuntikan ganda dengan interval 12 hari. Setelah 48 jam akhir perlakuan, sapi pada K1 dan K2 diinseminasi menggunakan semen beku fertil. Observasi berahi dilakukan setelah penyuntikan terakhir. Koleksi darah untuk pemeriksaan level estradiol dilakukan segera setelah inseminasi dilakukan sedangkan koleksi darah untuk pemeriksaan progesteron dilakukan pada hari ke-7 pasca-inseminasi. Level steroid diukur menggunakan teknik enzyme-linked immunosorbent assay (ELISA). Pemeriksaan kebuntingan dilakukan 90 hari pasca-inseminasi menggunakan teknik palpasi rektal. Seluruh sapi menunjukkan gejala berahi setelah perlakuan. Level estradiol dan progesteron pada K1 vs K2 masing-masing adalah 294,98±110,48 vs 392,76±11,6 pg/ml (P>0,05) dan 23,85±15,14 vs 12,69±5,64 ng/ml (P>0,05). Persentase kebuntingan pada K1 vs K2 masing-masing adalah 60,0 vs 0,0%. Dari hasil penelitian disimpulkan bahwa metode presynch-ovsynch tidak dapat meningkatkan level steroid tetapi dapat meningkatkan persentase kebuntingan pada sapi aceh.

Kata kunci: sapi aceh, level steroid, persentase kebuntingan, presynch-ovsynch

INTRODUCTION

Aceh cattle are one of the potential local beef cattle in Indonesia. The growth rate of local beef cattle is not as high as other cross-breed cattle, however, they show productivity and give maximum economic efficiency in limited conditions (Romjali *et al*., 2007). Nevertheless, their population tends to decrease (FAO, 1996 in Arum *et al*., 2013). Low reproductive efficiency including anestrus and difficulty in estrus detection contribute to the low productivity in the development of cattle farm in Indonesia (Herdis *et al*., 1999)**.** Artificial insemination (AI) was disturbed due to the non-simultaneous estrus happening and bad intensity of the estrus. Estrus synchronization was developed to overcome the difficulty of estrus detection.

The new synchronization program which does not require estrus observation is presynch-ovsynch (presynchronization-ovulation synchronization). Presynch protocol includes twice injections of prostaglandin F2 alpha (PGF2α) before the ovsynch induction (Navanukraw *et al*., 2004). Ovulation synchronization method used the combination of PGF2α and gonadotropin-releasing hormone (GnRH). GnRH injection applied in day-0 to induce follicular ovulation and to start a new follicular cycle, in day-7 PGF2α was injected to regress the corpus luteum. In day-9, the cows were re-injected with GnRH to induce ovulation of dominant follicle which had been recruited after the first GnRH injection. Artificial insemination was performed 16-24 hours later without identifying the estrus symptoms (Pursley *et al*., 1997; Efendi, 2015)**.** Presynch was estrus pre-synchronization protocol carried out in the beginning of estrus cycle to optimize GnRH response in order to enhance pregnancy rate compared to ovsynch method (Moreira *et al*., 2000; El-Zarkouny *et al*., 2004).

GnRH administration stimulates anterior pituitary to secrete follicle stimulating hormone (FSH) and luteinizing hormone (LH). Both of the hormones play important role in folliculogenesis and ovulation. The follicles further produce estrogen which manifest as the estrus symptoms (Hafizuddin *et al*., 2011). Estrogen

increases the sensitivity of female genital organs which is marked by changes in the vulva and mucous production (Lammoglia *et al*., 1998; Andriyanto *et al.*, 2015). Lyimo *et al*. (1999) stated that estrus intensity correlated with estradiol level in blood with the correlation value of 0.7. Good estrus intensity indicates a success pregnancy. Therefore, the elevation of estradiol level due to presynch-ovsynch treatment was expected to be a predictor of AI success and increment of pregnancy rate. Otherwise, high LH concentration produces ovulation or luteinization in dominant follicle resulting in progesterone elevation in line with growth and formation of accessories corpus luteum (Pursley *et al*., 1997). Progesterone prevents early embryonic death, thus increasing the pregnancy rates (Willard *et al*., 2003).

Several synchronization protocols had been reported to be used in Aceh cows (Efendi *et al*., 2015; Siregar *et al*., 2015). Efendi *et al*. (2015) reported ovsynch protocol whereas Siregar *et al*. (2015) reported estrus synchronization protocol using progesterone (CIDR-B) and PGF2α. There has been yet any report on the implementation of presynch-ovsynch protocol in Aceh cows prior to this study.

MATERIALS AND METHODS

Research Method

Ten non-pregnant cows aged 1.5-3.0 years old, with a minimum of two months post-partum, and clinically healthy Aceh cows were divided into two groups of five cows each. First group (K1) was treated with presynch-ovsynch method. Presynch method started in day-0 with first injection of 5 mL PGF2α (Lutalyse^{TM}, Pharmacia & Upjohn Company, Pfizer Inc.) intramuscularly, after 14 days was followed by second injection of PGF2α, and was further followed by ovsynch protocol 11 days later. Ovsynch protocol began with the injection of 1 ml GnRH followed by the injection of 5 ml PGF2α 7 days later, and followed by the injection of 1 mL GnRH (Fertagyl^{TM} Merck, Animal Healthy) 48 hours later. After 12-24 hours, cows were inseminated with frozen semen and repeated 6 hours later. The motility of frozen semen used was more than 40%. The second group (K2) was estrus synchronized using 5 mL of PGF2α, injected intramuscularly, administered twice with a 12-day interval followed by artificial insemination 48 hours later. Pregnancy diagnostic was established on day-90 post insemination by rectal palpation technique.

Blood Collection and Preparation

Blood collection for estradiol examination was done immediately after the artificial insemination while blood collection for progesterone measurement was carried out in day-7 post-insemination. Blood serum was collected from cow's jugular vein using 5 ml disposable syringe and then inserted into a reaction tube and further stored in an ice box. Blood samples were then carried to the laboratory for serum collection. Tube was tilted 45 degrees and kept in 4° C and was subsequently centrifuged (1200 g) for 10 minutes. Serum was collected using micropipette and stored inside a micro tube in freezer with temperature -20° C.

Estradiol and Progesterone Concentration Measurements

Steroid hormone measurement was carried out using the enzyme linked immunosorbent assay (ELISA) method using DRG progesterone/estradiol ELISA kit (DRG Instrumenst GmbH, Germany). Each ELISA well (well micro titer plate) was filled with 25 µL of standard solution, sample, and control solution, then incubated for 5 minutes at room temperature. Subsequently, each well was added and mixed with 200 µl conjugate estradiol/progesterone-HRP reagent and then incubated for 6 minutes at room temperature and then shaken rapidly to remove the contents of the well. The next procedure was flushing the plates with ELISA washer three times by adding 400 µL washing solution in each well. After that, each well was added with 200 µl substrate solution and incubated for 15 minutes in room temperature. The enzymatic reaction was stopped by adding 100 µL stop solution into each well. Absorbance values were read by ELISA reader in 10 minutes with absorbance of 450 nm.

Data Analysis

Steroid levels were analyzed using t-test, whereas estrus and pregnancy percentage were reported descriptively.

RESULTS AND DISCUSSION

The research results are presented in Table 1. There was no difference of estrus percentage between K1 and K2 at 100.0%. Estrus symptoms were in accordance with symptoms reported by Sonmez *et al*. (2005). Estradiol level at the moment of artificial insemination in K1 and K2 were 294.98±110.48 and 392.76±11.6 ng/mL respectively (P>0.05), whereas progesterone level in K1 and K2 on day-7 post-insemination were 23.85±15.14 and 12.69±5.64 ng/mL respectively (P>0.05). The pregnancy percentage of K1 and K2 were 60.0% and 0.0% respectively. The estrus response of Aceh cows after being induced by synchronization method was relatively effective. Siregar *et al*. (2015) also reported the high estrus response in cows induced

Table 1. Estrous response, steroid level, and pregnancy percentage of Aceh cows undergoing either presynch-ovsynch or PGF2α induction protocol

Parameter	Presynch-ovsynch (K1, n= 5)	PGF2α (K2, n= 5)
Estrous response (%)	100.0	100.0
Estradiol level (pg/mL) during artificial insemination	294.98±110.48	392.76±11.6
Progesterone level (ng/mL) in day-7 post artificial insemination	23.85±15.14	12.69±5.64
Pregnancy percentage (%)	60.0	0.0

by estrus synchronization using CIDR-B as well as PGF2α was 100.0% each.

Estradiol concentration of Aceh cows in this research (294.98±110.48 to 392.76±11.6 pg/mL) was higher than that reported by Domènech *et al*. (2011) (26.75±8.63 to 52.91±12.99 pg/mL in Holstein cows) and Naik *et al*., (2013) (20.24 pg/mL in Punganur cows). When compared with the concentration of estradiol in the same breed, this result was relatively higher than that reported by Siregar *et al*. (2016) and Thasmi *et al*. (2017). Siregar *et al*. (2016) reported estradiol concentration of Aceh cows in estrus period was 223.13±9.50 pg/ml whereas Thasmi *et al*. (2017) reported estradiol concentration between fertile cows and cows with repeated breeding at the estrus period were 110.4±20.1 and 101.6±17.4 pg/mL respectively. Indonesia local cows tend to have higher estradiol level than other breed. Airin *et al*. (2014) informed that estradiol concentration of Bali cows at the moment of estrus period was as high as 107.77±55.94 pg/mL.

No significance difference (P>0.05) of estradiol concentration between K1 and K2, which was in accordance with Pulley *et al*. (2013) which reported that there was no difference of estradiol concentration between control and cows induced by equine chorionic gonadotropin (eCG) hormone. This hormone functionally has similar activity with GnRH. Akbari *et al*. (2011) also reported that the difference of estradiol concentration between Holstein cows induced either by presynch-ovsynch or presynch-heatsynch was not significant. Ribeiro *et al*. (2012) revealed that presynch protocol did not influence estradiol concentration at the time of artificial insemination with concentration in double ovsynch and presynch protocol were 6.4 and 5.8 pg/ml, respectively.

From the data above, even though there were no data comparing estradiol concentration between cows induced by PGF2α and presynch, it can be expected that GnRH treatment was not able to elevate estradiol concentration. Follicular phase occurred in a very short time that time difference of blood collection allows different measurements results. Blood collection performed during artificial insemination allowed errors in determining the estradiol peak level.

Progesterone concentration of Aceh cows in this study was higher compared to the progesterone concentration reported by Siregar *et al*. (2016) and Arimbawa *et al*. (2012). Progesterone concentration of Aceh cows throughout one cycle that was revealed by Siregar *et al*. (2016) in day-13 until day-16 of estrus cycle was 0.10±1.67 ng/mL. Progesterone levels of Bali cows in day-7 and day-8 of estrus cycle were 3.95±0.04 and 5.48±0.06 ng/mL, respectively (Arimbawa *et al*., 2012). The mean progesterone concentration of coastal cows in the luteal phase undergoing ovulation induction with ovsynch method was 6.684 ng/mL (Zumarni, 2012). Additionally, progesterone concentration in this study was also higher compared to the report by Amiruddin *et al*. (2013) in similar breed cows. The mean progesterone concentration of Aceh cows in day-7 of estrus cycle undergoing superovulation induction by FSH was 17.40±5.84 ng/mL. The elevation of progesterone level in this research was possibly due to the administration of GnRH which caused accessories corpus luteum formation which resulted in the drastic increase of progesterone concentration than usual (Pursley *et al*., 1997). Stevenson *et al*. (2004) conveyed that GnRH administration induced estrus, LH surge, ovulation, and normal corpus luteum formation in dairy cows.

Even though there was no statistically significant difference of progesterone concentration between K1 and K2 (P>0.05), the progesterone concentration of K1 was still higher than K2. This was caused by the double administration of PGF2α with 14-days interval in K1 which in turn able to give optimal response to GnRH from ovsynch induction (Moreira *et al*., 2000; El-Zarkouny *et al*., 2004). Vasconcelos *et al*. (1999) revealed that the success of ovsynch depends on the protocol usage at the beginning of the cycle. The use of protocol between day-5 to day-12 of the cycle (initial luteal phase) increased fertilization, therefore increasing pregnancy rates. Progesterone concentration in both groups were above average >5 ng/mL which means they were above the basal level (Appavu and Holtz, 1992). The concentration indicated that corpus luteum was in functional phase. It was because from day-4 the progesterone concentration increased gradually and reached its peak in the middle of the estrus cycle (Akusu *et al*., 2006).

Pregnancy percentage of Aceh cows in K1 was increased significantly than K2 (60.0% vs 0.0%). Pregnancy percentage of Aceh cows with different synchronization method had been reported by Siregar *et al*. (2015) and Efendi *et al*. (2015). Siregar *et al*. (2015) reported that pregnancy percentage of Aceh cows undergoing synchronization protocol using PGF2α and progesterone were 80.0% and 80.0% respectively, whereas Efendi *et al*. (2015) revealed that pregnancy percentage of Aceh cows undergoing synchronization protocol using ovsynch and PGF2α were 80.0% and 60.0%, respectively. The differences of study results were possibly due to the different treatment time. This study was done in May until June when temperature and humidity in Aceh Province reached its highest peak that causing environmental heat stress and affecting the number of conception. The increased pregnancy percentage in K1 compared to K2 (60.0% vs 0.0%) was probably because the GnRH administration prevented early embryonic death and increased the pregnancy rate. Increased pregnancy rate correlated with GnRH ability to elevate progesterone concentration through accessories corpus luteum formation (Pursley *et al*., 1997). Yildiz (2010) reported that GnRH administration in day-9 post-insemination could increase pregnancy number compared to control group (77.7% and 50.0% respectively). Moreira *et al*. (2000) revealed that the pregnancy rate of cows induced with presynch-ovsynch method was 12% higher that cows undergoing only ovsynch induction.

CONCLUSION

Presynch-ovsynch method improved pregnancy percentage but did not elevate the steroid level in Aceh cows.

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