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EFFECT OF PROGESTERONE AND HCG AFTER ARTIFICIAL INSEMINATION ON PREGNANCY RATES AND PROGESTERONE LEVELS IN BALI TIMOR COWS

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ABSTRACT

This study determined whether the administration of progesterone and hCG after insemination increased pregnancy rate in Bali Timor cows. A total of 30 Bali Timor cows (BW ± 200-225 kg; aged 3-7 years old) with different parity were used in this study. Cows were inseminated with progesterone and hCG. Cows were grouped based on body weight and number of parities following a Complete Randomized Design with 3 treatments and 10 replications. The treatments were: P1 = did not receive any treatment (control; n=10); P2 = estrus induced by PGF2α 125 mg on day 5, 7 and 9 (n = 10); and P3 = estrus induced by hCG 1000 IU on day 5 after artificial insemination (AI; n=10). Blood samples were collected from the jugular vein of the animals on day 0 and continued for a period of 33 days after AI using 5 mL heparinised pre-set syringes Blood samples were then centrifuged at 1500 rpm for 10 minutes. Serum was harvested and stored at -20oC until serum concentrations of progesterone and hCG were determined using ELISA procedures. Pregnancy was determined by rectal palpation. The results showed that the AI pregnancy rates was greater in group treated with hCG (70%) than cows induced with progesterone (50%) or the control group. The concentrations of both progesterone and hCG were increased simultaneously on day 5 to 11 post AI. In conclusion, progesterone and hCG application in Bali Timor cows after artificial insemination could increase pregnancy rates.

Keywords: progesterone, hCG, artificial insemination

INTRODUCTION

Bali Timor cattle plays an important role in the livelihood of small holder farmers in East Nusa Tenggara Province as an asset. Although Bali Timor cattle in this area has contributed significantly to the national beef consumption especially in DKI Jakarta province, which accounted for 60,000-70,000 heads per year of shipments, the population is still low. As a strategy to increase the number of populations of this animal, the government imposed the program of "Upaya Khusus Sapi Indukan Wajib Bunting (UPSUS SIWAB) since 2018 with the Artificial Insemination (AI) technology. However, the success of this program is still relatively low, which is about 40% to 50%. This is caused by the failure of the embryonic development and most commonly due to the failure of the embryo to prevent luteolytic and maintain pregnancy (Sreenan and Diskin, 1986).

In early pregnancy, the embryo inhibits oxytocin to stimulate the release of PGF2α by producing a protein, interferon-π, which works locally in the uterus to inhibit PGF2α secretion and thereby preventing luteolytic and consequently progesterone is secreted to support the survival of the embryo (Mann *et al*., 1998b). Progesterone is secreted by the corpus luteum (CL) which plays a critical role in regulating the histotrophic environment in supporting embryonic development. The important role of progesterone is to promote embryonic development, stimulate the secretion of interferon (INF-π) (Mann and Lamming 2001). Stronge *et al*. (2005) grouped 7 groups of 871 inseminated cows and found a linear and quadratic relationship between the concentrations of milk progesterone on days 5, 6, and 7 after insemination and progesterone increase between days 4 and 7 with embryonic survival.

Human chorionic gonadotropin (HCG) is a luteotropic hormone, which maintains the corpus luteum. The use of HCG (Santos *et al*., 2001) after artificial insemination will increase

progesterone secretion as well as induce accessories corpus luteum (Fonseca *et al*., 2001) thereby increasing progesterone concentration, and also affecting conceptus. Previous study has shown that HCG administration on cattle 5 days post-insemination had increased conception rate and progesterone concentration during the early critical phase of pregnancy (Akhtar *et al*., 2018). However, there is limited information on the use of progesterone and HCG on Timor Bali cattle in preventing early embryonic loss during early stages of pregnancy. The objective of this study was to determine whether the administration of progesterone and hCG after insemination increased pregnancy rate in Bali Timor cows.

RESEARCH METHODS

Animal Handling and Site of Study

This study was undertaken in Nunfae Village, Central Fatuleu Subdistrict, Kupang Regency from May to September 2021. A total of 30 Bali Timor cows (BW ± 200-225 kg; aged 3-7 years old) in healthy body conditions with different parity were used in this study. The animals were maintained in a grazing system on communal pastures and supplemented with 2 kg of feed consisting of bran (90%) and fish meal (10%) per head per day.

Experimental Design

In this study, 30 cows were grouped by body weight 200-225 kg with number of parity 1-2 following a Complete Randomized Design with 3 treatments and 10 replicates. The treatments were: P1 = did not receive any treatment (control; n = 10); P2 = estrus induced by PGF2α 125 mg on day 5, 7 and 9 (n = 10); and P3 = estrus induced by hCG 1000 IU on day 5 after AI. Cows were synchronization using the hormone GnRH (100 µg) on the 0th day (d-10), then on the 7th day (d-3) injected PGF2α i.m (25 mg), and then 10th day re-injected GnRH (d-1) at a dose of 100 µ in accordance with the Ovsynch protocol. Estrous was observed twice daily (morning and evening) after treatment for 5 consecutive days. Estrous observation included symptoms such as the condition of vulva (swelling, wet, and red), transparent mucus that comes out hanging through the vulva, riding and silently ridden by other cow, as well as other symptoms such as unsettled and lack of appetite. Frozen semen from Angus bull was used in this study for the AI. The semen was evaluated the motility before the AI in accordance with the Indonesian National Standard. The AI was conducted 12 hours after the estorus symptoms. Cows were injected i.m with 125 mg/head of progesterone (Potahormon®, 1 ml = 6.25 mg progesterone on days 5th, 7th, and 9th after AI (P2) or i.m 1000 IU of hCG (Ovigil) on the 5th day after AI (P3). Conception rates were calculated based on the number of pregnant cows at each diagnosis (palpation per rectal) divided by the number of cows previously inseminated and treated and multiplied by 100%. Concentrations of progesterone on days 0 to 33 after AI.

Sampling and Hormone Measurement

Blood samples were obtained on the day of AI, and were twice weekly for 4 weeks. Blood samples were collected from the jugular vein of animals on day 0 and continued for a period of 33 days after AI using 5 mL heparinised pre-set syringes Blood samples were then centrifuged at 1500 rpm for 10 minutes. Serum was harvested and stored at -20oC until serum concentrations of progesterone and hCG were determined using ELISA procedures. Progesterone concentrations were measured according to the ELISA procedures provided with the kit manual. 25 µl of the standard, sample, and control solutions, were filled into each well microplate and the added with the 200 µl of the enzyme conjugate progesterone into each well of microplate and homogenized for 10 seconds to ensure the solutions were completely mixed. Incubation was the carried out for 60 minutes at room temperature. The microplate was shaken quickly to remove the contents of the wells. The wells were then 3 times by adding 400 µl of wash solutions in each well. Microplate was tapped quickly on absorbent paper to remove the remaining droplets from the wells. The 200 µl substrate solution was added to each well and the incubated for 15 minutes at room temperature. Enzymatic reactions were stopped by adding 100 µl stop solutions at each well. The absorbance value was read on a microtiter plate reader with a wavelength of 450±10 nm within 10 min.

Statistical Analysis

The rate of pregnancy by treatment was compared using chi-square analysis, while the

difference in progesterone concentrations between treatments was determined using general linear model procedures by SAS.

RESULTS AND DISCUSSION

Pregnancy Rate of Cows after Artificial Insemination

Administrating cows with progesterone had a significant effect (P<0.05) on conceptus loss during early pregnancy of Timor Bali cows. This was due to the increase of progesterone concentrations, which was able to increase the levels of interferon-π to stop the development of oxytocin receptors that will induce the synthesis of PGF2α resulting in luteolysis and embryo loss (Mann *et al*., 1998a; Mann *et al*., 1998b). Interferon-π secreted by the trophectoderm of conceptus which is the primary signal for the introduction of maternity, initiating the process of blocking luteolytics and preventing the death of the corpus luteum (Meyer *et al*., 1995).

The results showed that the percentage of treatment P3 (70%) differed markedly compared to P2 (50%) and compared to control P1(30%). In addition, the difference between P2 and P3 explains that injection of hCG on the 5th day after AI was able to maintain the functional corpus luteum formed, and therefore progesterone still produced to maintain the early pregnancy. Synchronization of ovulation performed before AI can cause various sizes of follicles (Santos *et al*., 2010), and induce ovulation in small follicles resulting in the formation of a small corpus luteum thereby lowering progesterone concentration during the next phase (Souza *et al*., 2007).

Tabel 1. The results pregnancy of cows after the artificial insemination

Group	Total of cows	Total pregnancy of cows (head)	Presentation of Gestation
P1	10	3	30[a]
P2	10	5	50[b]
P3	10	7	70[c]

Values with different lowercase superscript differ between lines (P<0.05).

Similar finding was reported by Starbuck *et al*. (2001), but in not in accordance with other studies (Shams-Esfandabati dan Shirazi, 2007; Morris dan Diskin, 2008; Monteiro *et al*., 2014). The difference between each study could be related to some factors including the type of the animals used, feeding as well as maintenance management. Progesterone injection to the animals with low post-ovulation progesterone concentrations was aimed to create an embryotropic environment for the embryo to grow, develop, and survive until it is able to prevent luteolytic by the secretion of luteotropin IFN-π (Mann and Lamming, 2001). Furthermore Mann *et al*. (2006) reported that trophoblasts was increased 4 times coincide with 6 times increased in uterine concentration of IFN-π in non-lactating cows given progesterone between days 5 and 9 compare with cows when treated between days 12-16. Progesterone functionally could be mediated by a direct influence on the embryo or an indirect influence through the uterus or both.

Pregnancy in cows depends on the effective functioning of the endocrine communication system between the cow and the embryo, thereby the cow has to maintain the corpus luteum and then become pregnant or undergo luteolytic and ovulate again. Bali Timor cows used in this study were able to increased progesterone concentrations and hCG after AI (Figure 1). These results explain that increased concentrations of progesterone and hCG in early pregnancy plays an important role in preventing luteolytic of corpus luteum and maintained the pregnancy. An important component of the luteolytic mechanism is the development of oxytocin receptors in the epithelial luminal of the endometrium that cause oxytocin to urge a stimulatory action against PGF2α secretion. During early pregnancy in cow, embryos inhibit oxytocin stimulate the release of PGF2α by inhibiting the development of oxytocin receptors in epithelial luminal (Mann *et al*., 1998a) and through induction of prostaglandin inhibitor synthesis.

The embryo produces a protein, interferon-tau (interferon-π), which works locally in the uterus to inhibit PGF2α secretion. To prevent luteolytic, the embryo has to develop well enough to secrete enough interferon-π that will prevent PGF2α secretion. Poor embryonic development is associated with low interferon-π proteins, resulting in luteolytic and embryo loss (Mann *et al*., 1998b). Progesterone is a vital hormone for the success of pregnancy in ruminants (Spencer *et al*., 2007). Progesterone plays an important role in stimulating the production of various endometrial secretions that are essential for successful embryonic development (Geisert *et al*., 1992). The profile of progesterone hormone measured at the time of artificial insemination is listed in Figure 1 progesterone concentration increases per day after IB until it reaches its peak on day 24th.

The treatment of HCG on the 5th day after artificial insemination substantially increases progesterone concentration for at least the next 2 weeks. This is due to the addition of hCG will lead to luteinization of large follicles (Price and Webb, 1989) present at the time of treatment. Ireland *et al*., (1980 observed that corpus luteum of each of four beef cows were formed as a result of corpus luteum induction when the animals treated with same treatment and the ovaries were collected from the slaughterhouse on the 12th day of the estrus. Therefore, hCG induction in cows on day 5th after artificial insemination is an appropriate technique for stimulating progesterone concentrations during the critical phase in early pregnancy and it is worth for large experiment using cows with compromised fertility to determine whether it will be effective in increasing pregnancy rates in field conditions (Robinson *et al*., 1989).

The Profile of Progesterone in Cows After Artificial Insemination

In the present study, the progesterone concentrations increased on day 9th to 11th after AI. The increased concentration of hCG (days 9-11) in this study indicated that the corpus luteum was formed to produce progesterone at sufficient concentrations to maintain early pregnancy. Akhtar *et al*. (2018) found a higher conception rate (50%) in¬¬ crossbred Frisian x Sahiwal cows a dose of when the animals treated with hCG of 3300 IU i.m. on the 7th day after AI compared with progesterone treatment. On the other hand, treating cows with hCG caused higher corpus luteum of the cows which accounted for an 80%. The development of the corpus luteum during the estrus cycle phase was in accordance with other studies using GnRH (Vasconcelos *et al*., 1999) and HCG (Stevenson *et al*., 2007).

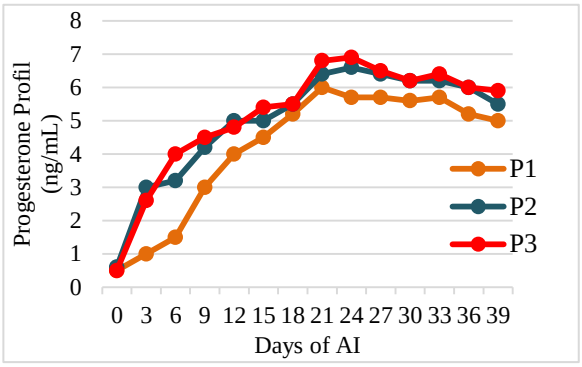

Figure 1. Profile of Progesterone after Artificial Insemination

The results of statistical analysis of progesterone injection on days 5, 7, and 9 after AI affects the concentration and progesterone profile from day 5 to day 9 after AI. However, there was no significant effect of treatment on progesterone concentration on day 11 to day 27 after AI. Progesterone profile on days 11 to 27 may affect the conception rate in P2 (50%). This Could be related to the function of corpus luteum in secreting progesterone to maintain pregnancy. Furthermore, the concentrations of progesterone P1, P2 and P3 increased gradually on the 9th day as shown in Fig.1. The profile or pattern of progesterone in this study was the same as the Monteiro *et al*. study (2014) with a profile in cows albeit of different magnitudes.

Villarroel *et al*., (2004) reported that a lower concentration of progesterone in cows with low fertility after 6 days of estrous. In addition, cows exhibit a delay in increase of progesterone post ovulation on the 16th day after insemination had underdeveloped embryos and producing a slight interferon-π. Conversely, in cows that experience an initial increase in progesterone post ovulation had more embryonic lengthening and produce interferon-π in large quantities (Mann and Lamming, 2001). Moreover, cows treated with progesterone from day 2-5 showed 10 times an increase in the lengthening of the conceptus on the 14th day (Garret *et al*., 1988).

CONCLUSIONS

Injecting Bali Timor cows with i.m progesterone 125 mg after AI on the 9th and i.m hCG 1000 IU 5th day after AI increases pregnancy rates

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