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ABSTRACT. The objective of this study was to evaluate the breeding performance of Indonesian beef cattle (Ongole cross) as recipients for embryo transfer using Limousin embryos. As a result, the pregnancy rate was 35% (7 out of 20 cows). There was a significant difference ($P<0.01$) in the serum progesterone concentration between the nonpregnant and pregnant cows at the time of the embryo transfer (day 7 after the estrus). The pregnancy rate in the Indonesian beef cows was low, which may be due to their insufficient genetic quality and/or low physical conditions caused by the poor management, like a low-nutrition diet. The low progesterone concentration in the nonpregnant cows on day 7 may be associated with the failure of embryo implantation.

KEY WORDS: embryo transfer, Indonesian beef cattle, pregnancy rate, progesterone

The increased production of high-quality beef is required to meet the demand of the big population in Indonesia, but there has always been a gap between supply and demand of beef, with national beef production only satisfying less than half of demand [5]. The beef cattle breed mainly kept in Indonesia is Ongole cross, followed by Simmental, Limousin, and the native cattle like Madura breed. The weight and quality of Ongole cross's carcass are not so good; therefore, the numbers of Limousin and Simmental cattle, as beef cattle, are expected to increase in Indonesia. Most beef cattle are mated naturally, whereby a bull is released into a cowherd approximately six weeks after calving period. In some cases, beef cattle can also be bred through artificial insemination. Natural mating and artificial insemination are not effective to increase the numbers of superior beef cattle, such as Limousin and Simmental cattle; whereas embryo transfer (ET) can directly produce such beef cattle if a sufficient number of Ongole cross cows are utilized effectively as recipients for ET. The problems associated with beef production in Indonesia strongly require collaborative research with some policy makers and stakeholders, including central and local governments, investors, and cooperative and farmers' communities. It is necessary to break through the plateau in the beef production system in Indonesia using ET technology.

It is generally accepted that ET programs constitute an important approach to rapidly multiply genetically superior animals, and consequently, improve livestock production [3]. The success of ET depends on factors associated with the embryo, the recipient, or an interaction among factors of the embryo and recipient, such as morphologically poor quality of embryos, inconsistency of the estrous cycle of the donors with that of the recipients, nutrition of recipients, high temperature environments, and luteal insufficiency in recipients [1, 2, 6, 9–11].

Progesterone (P_4) is the principal hormone responsible for the maintenance of pregnancy. The synchrony between the uterine status and embryo development is important for maximizing embryonic survival and chances of pregnancy maintenance, and it is partially influenced by the ability of P_4 to modulate both the uterine environment and embryo growth [3, 4, 8]. However, plasma or serum P_4 levels during ET were related to the pregnancy rate to some extent in some studies [7, 10], while others found no relation [8, 11]; thus, the contradictions in the arguments may be due to the interaction of these factors. Therefore, it may be necessary to evaluate the effect of P_4 concentration under the same management and environmental conditions, especially on the cattle herd in a tropical environment like Indonesia.

The objectives in the present study were to evaluate the breeding performance of Indonesian beef cattle (Ongole cross) as recipients for ET and to compare their breeding performance with that of dairy cows (Holstein-Friesian) evaluated in the previous study [4].

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The use of animals in the present study was approved by the Animal Ethics Committee of the Faculty of Veterinary Medicine, Airlangga University, Surabaya, Indonesia. The experiment was conducted in a local government farm in Tuban, East Java, Indonesia. Twenty Ongole cross cows reared in the same station at in the second parity (60 to 90, mostly 90 days after the last parturition) were selected based on the general healthy condition, body condition score (BCS; 2.5 to 3 out of 5 scores, 2.75 ± 0.26 as mean ± standard deviation) [12]. Normal reproductive organs and ovaries with normal corpus luteum (20 mm or more in diameter) were examined by an ultrasound device. All the beef cows were maintained under the same management. They were housed in well-ventilated roofed sheds with an anti-slippery cemented floor. They were fed with fresh grasses (mainly 30 kg/day King grass) and concentrated diet (18% crude protein) twice a day at about 9:30 A.M. and 3:30 P.M. with clean drinking water and mineral blocks provided *ad libitum*.

Estrus of all the cows was synchronized by 25 mg of an intramuscular injection of dinoprost tromethamine (prostaglandin $F_{2\alpha}$; Lutalyse®, Zoetis, Parsippany, NJ, U.S.A.) twice at 11-day intervals according to the manufacturer's protocol. Estrus signs were observed in the morning and afternoon twice a day. Estrus was confirmed by the mucous and cervix dilatation using a vaginoscope. The day when estrus signs were observed was interpreted as day 0. The cows were implanted on day 7 with thawed frozen-stored embryos (Limousin breed), which were purchased from Balai Embryo Ternak Cipelang (West Java, Indonesia), using a general ET technique. During ET, the cows were administered intramuscularly with 1,500 IU of human chorionic gonadotropin (Chorulon®, Intervet, Boxmeer, Netherlands). A rectal examination was done on day 90 to judge pregnancy. The difference of the pregnancy rate between the Ongole cross beef cows and Holstein-Friesian dairy cows was statistically analyzed using the Chi-square test and odds ratio. Differences with $P<0.05$ were considered statistically significant.

Whole blood samples were collected from jugular vein of the cows at the onset of estrus (day 0), at the time of the ET (day 7), and on day 21. Serum was separated from the whole blood by centrifugation. Serum progesterone concentration was measured by an enzyme-linked immunosorbent assay using an analyzer (DRG:HYBRiD-XL®, DRG Marburg, Germany) and a kit (17-OH Progesterone, DRG). Data were shown as mean ± standard deviation. Statistical analyses were performed using the Mann-Whitney *U* test. Differences with $P<0.05$ were considered statistically significant.

In the present study, 7 out of 20 beef cow recipients got pregnant. Based on this result, the pregnancy rate was 35%. All the calves (three male and four female) born by ET were normally healthy, weighing 32 kg on an average. One of the Limousin calves and one of Ongole cross dam are shown in Fig. 1.

The serum progesterone concentration (*ng*/m*l*) was 0.43 ± 0.39, 2.84 ± 1.70, and 2.59 ± 1.05 on days 0, 7, and 21, respectively, in the nonpregnant beef cows, whereas 0.39 ± 0.55, 7.06 ± 3.98, and 13.7 ± 6.40 on days 0, 7, and 21, respectively, in the pregnant beef cows (Fig. 2A). There was a significant difference in the progesterone concentration between the nonpregnant (n=13) and pregnant beef cows (n=7) on days 7 ($P<0.01$) and 21 ($P<0.001$).

Before the present study, the same research group performed an experiment using Holstein-Friesian dairy cows (60 to 90 after the last parturition) as recipients for ET using Simmental embryos purchased from the same company with the same materials and protocol as those in the present study [4]. Based on the data of the previous experiment, the pregnancy rate was 55% (11 out of 20 dairy cows), which is high compared with that (35%, 7 out of 20 beef cows) in the present study. However, there was no significant difference ($P=0.204$ on the Chi-square test and $P=0.124$ on the odds ratio) of the pregnancy rate between these two types of cows probably due to a small number of animals. The change in the progesterone concentration in the dairy cows (Fig. 2B) was similar to that in the beef cows in the present study (Fig. 2A). The serum progesterone concentration (*ng*/m*l*) was 0.44 ± 0.40, 3.53 ± 2.06, and 2.44 ± 1.05 on days 0, 7, and 21, respectively, in the nonpregnant dairy cows, whereas 0.19 ± 0.45, 6.64 ± 3.87, and 18.7 ± 11.7 on days 0, 7, and 21, respectively, in the pregnant dairy cows. There was a significant difference in the

Fig. 1. An Ongole cross dam (A) used as a recipient for embryo transfer and a Limousin calf (B) born by embryo transfer.

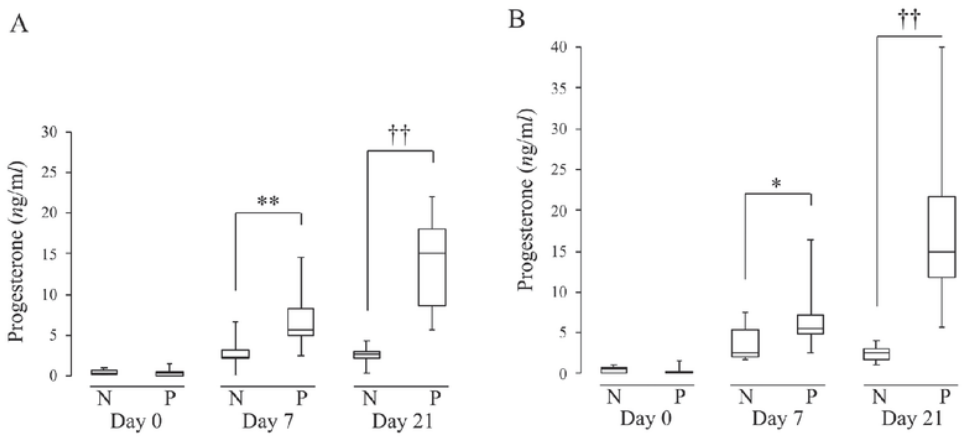

Fig. 2. Comparison of serum progesterone concentration between nonpregnant (N) and pregnant groups (P) in beef (A) and dairy (B) cows as recipients for embryo transfer. All data are represented as box and whisker plots. *P<0.05, **P<0.01, and ††P<0.001 between nonpregnant and pregnant groups with a statistical significance. Figure 2B was newly drawn using the data published previously by the same research group [5], and a statistical analysis was newly performed in the present study.

progesterone concentrations between the nonpregnant (n=9) and pregnant dairy cows (n=11) on days 7 (P<0.05) and 21 (P<0.001). The difference of the progesterone concentration between the nonpregnant and pregnant groups in the dairy cows was similar to that in the beef cows in the present study, although the pregnancy rate was high (55%) in the dairy cows compared with that (35%) in the beef cows, as mentioned above.

Successful implantation of ET depends on several factors, such as quality of embryos and recipient cows, and skills of operators [1, 2, 5, 6, 9–11]. The quality of embryos depends on the age of donors, types of embryos (fresh or frozen), freezing method, and contamination of infectious agents. The recipients should be generally healthy without any defect and infectious agent in the reproductive system, respond appropriately to the estrous synchronization, have enough forage, and have a moderate BCS of 2.8–3.5. The operators should transfer embryos precisely in the apex cornu of the uterus in the correct time and with less stress to recipient animals.

There could be a difference in the quality of embryos between the previous [5] and present experiments because Simmental and Limousin embryos were used in the previous and present experiments, respectively. However, the embryos were processed similarly by the same company. The skills of operators were the same between the two experiments because both were performed by the same research group. The biggest difference between the two experiments are the quality of recipient cows. Therefore, the difference in pregnancy rate between the two experiments can be attributed to the difference in breeding performance as recipients for ET between dairy and beef cows in Indonesia.

Holstein-Friesian (*Bos taurus*) dairy cows were used as the recipients for ET in the previous study [5], whereas Ongole (*Bos indicus*) cross beef cows were used in the present study. Embryos of Simmental and Limousin breeds (*Bos taurus*) were used in the previous and present experiments, respectively. The genetic difference between Ongole cross recipient cows and Limousin embryos may affect the pregnancy rate of Ongole cross recipient cows as a species barrier, although these two species can be routinely interbred in many areas of the world.

Another difference between dairy and beef recipient cows is physical conditions: BCS in dairy cows was moderate (3.50 ± 0.24; an author's personal information, TL) in the previous study [5], whereas BCS in beef cows was lower (2.75 ± 0.26) in the present study. This lower physical condition in beef cows is probably caused by the poor management, such as a low-nutrition diet, which may affect the response to estrous synchronization and the endocrine status, including the secretion of P_4. The insufficient secretion of P_4 in the beef cows on day 7 in the present study may be associated with the failure of embryo implantation. This result suggests that serum P_4 concentration plays an important role in embryo implantation to increase the pregnancy rate; some studies agree with this view [7, 10], while others disagree with it [8, 11].

Based on this assumption, methods to increase the pregnancy rate in Indonesian beef cows by ET should include selecting recipients with moderate BCS (e.g., 2.8–3.5) and/or implanting embryos in recipients with a sufficiently high concentration of P_4 (e.g., 7.06 ± 3.98 *ng*/m*l*) on the day of ET. Further studies are required to achieve an ideal pregnancy rate in Indonesian beef cows using ET.

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