Response of Bali cows on superovulation for in-vivo embryo production

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Abstract. The aim of this study was to know the response of Bali cows on superovulation for embryo production. A total of two Bali cows (BC1 and BC2) obtained and selected from smallholder farms were used in the present study. These cows were selected based on good general health, body condition score, and reproductive organ. The cow that absent of health disorder and body injury as well as had a body condition score of 6 or 7 were subsequently subjected for reproductive organ examination. The cow that did not have any reproductive organ disorder was then examined using ultrasound for ovaries. Those cows that have a number of follicles at the ovary(es) with or without corpus luteum were selected as donors. The cows were superovulated using hormones and inseminated artificially at a fixed time. Embryos were flushed at day-seven after insemination. Parameters measured were 1) response of Bali cows on superovulation treatment; 2) the number of embryos at each ovary; 3) the quality of embryos, and 4) embryo development stage. The results of this study showed that Bali cows were responded to superovulation. After first superovulation, BC1 produced five follicles; one in the left ovary and four in the right ovary. On the other hand, BC2 produced seven follicles; two in the left ovary and five in the right ovary. It can be concluded that Bali cows responded to superovulation.

Keywords: Bali cow, superovulation, embryo, follicle.

1. Introduction

Embryo transfer (ET) in cattle is the second generation of reproductive technology after artificial insemination (AI). ET is a tool of reproductive biotechnology that used to multiply the number of animals with high genetic value [1] and accelerates the improvement of animal genetic [2]. Furthermore, Paramio [3] stated that assisted reproductive technology such as AI and ET or multiple ovulation embryo transfers (MOET) has been used to increase the reproductive efficiency of the animals as well as accelerates their genetic value.

Basically, the main purpose of ET is to increase the reproductive value of the cows as well as to enhance the purpose of the cows during their live. This due to that some cows have a low reproductive rate and having too long generation interval [4]. This ET technology involves embryo production both in-vivo and in-vitro. In-vivo embryo production involves several stages such as superovulation (multiple ovulation; MO), insemination, and embryo flushing, and subsequently can be transferred directly in the form of a fresh embryo or preserve (freezing).
Superovulation is intended to increase ovulation rate and produce high-quality embryos that can be transferred to the other animal [5,6]. However, there are several obstacles to the application of this technology. Several differences in animal responses on the variation of embryo production after superovulation are affected by some factors including types of hormonal used [7], vitamin [8], as well as the species of the donor.

In Indonesia, especially in South Sulawesi Province, approximately 1.4 million cattle raised by smallholder farms. Bali cattle are the most raised in this region; in many reasons that this species is easy to manage by the farmers, has good reproductive performance and a high percentage of a carcass. However, for a few decades, many Bali cows are mated using reproductive technology of artificial insemination (AI). In this case, the straws for AI are mainly from the bulls of the other species such as Simental, Limousine, Brahman, etc, and very few using the straws of Bali bull.

So far, the development of Bali cattle for embryo production has not been well conducted. This study is intended to try the reproductive technology of superovulation for the future development of embryo production especially in Bali cows. This is a preliminary study whereas superovulation is conducted in Bali cows. Therefore, this study aimed to know the response of Bali cows on superovulation for embryo production.

2. Materials and Methods

2.1. Animals and Donor selection

This study was conducted in the Laboratory of Animal Reproduction Faculty of Animal Science Hasanuddin University farm. The animals used in the present study were Bali cows obtained from smallholder farmers. Selection and determining Bali cows for donors were conducted together with the farmers. There were two selected Bali cows for the donor that was not pregnant and had parity one to three.

2.2. General health examination, scoring body condition, and reproductive organ examination of the donor

In order to select two donors of Bali cows, the cows were examined for general health, body condition score, and reproductive organ.

2.2.1. Health examination and scoring the body condition, an examination of general health and body condition of the Bali cows candidate were conducted at each cow. The cow that absent of health disorder and body injury as well as had a body condition score of 6 or 7 were subsequently subjected for reproductive organ examination.

2.2.2. Reproductive organ examination, an examination of the reproductive organ was performed to all donors candidate such as vulva, vagina, cervix, uterine, and ovaries by palpation per rectum. The cow that did not have any reproductive organ disorder was then examined using ultrasound for ovaries. Those cows that have a number of follicles at the ovary(es) with or without corpus luteum were selected as donors. The cow that suffered from an ovarian disorder such as anestrus or cyst was not selected for the donor.

2.3. Superovulation and artificial insemination (AI)

Prior to superovulation, the Bali cows' donor was kept in the housing for adaptation. This due to that these cows was raised in the small farm extensively by the farmers. During housing, the cows were subjected to exercise in the barn and fed forage and rice bran to fulfill their nutrient requirement. Drinking water was prepared ad-libitum. Superovulation and artificial insemination (AI) of Bali cow donor was performed according to Bello [9] (Figure 1).
2.4. Embryo collection
The cows that superovulated and artificially inseminated were then collected their embryos on day-seven after insemination using the flushing technique. The embryos were grading according to their quality.

2.5. Parameter of the study
Parameters measured in the present study were 1) response of Bali cows on superovulation treatment and 2) the number of embryos at each ovary.

2.6. Data analysis
Data obtained in this study were tabulated using the Excel program. The response of each cow on superovulation was compared and analyzed descriptively. Responses to the superovulation of the Bali cows are compared the previous studies.

3. Results and discussion
After superovulation, both Bali cows used in this study appeared to be responded to the protocol. There was a change in the ovaries of the Bali cows. Each ovary was developed and became larger than usual. The development of ovaries was due to the increasing number of matured follicles. Table 1 shows the number of follicles at each ovary of both Bali cows prior to flushing.

| Table 1. Number of follicles at each ovary at first superovulation in Bali cows |
|------------------------|------------------------|------------------------|
| Bali cow code | Ovary | Left | Right | Total |
|-----------------|--------|------|-------|-------|
| BC1             |        | 1    | 4     | 5     |
| BC2             |        | 2    | 5     | 7     |
| Total           |        | 3    | 9     | 12    |

Table 1 shows that after first superovulation, BC1 produced five follicles; one in the left ovary and four in the right ovary. On the other hand, BC2 produced seven follicles; two in the left ovary and five in the right ovary. This indicated that Bali cows responded to superovulation. This result was in line with the statement of Armstrong [5] and González et al. [6]. They stated that the superovulation technique is intended to increase the number of ovulation as well as can produce quality embryos and can be transferred to the other animals. However, the response of Bali cows on superovulation in the present study appeared to be lower than the results reported by several studies.
Holm and Cellesen [10] stated that there are differences in-vivo embryo production at different breeds.

The technique for multiple ovulation in embryo production and transfer embryo has become important in breeding technology that can be applied to accelerate the achievement of genetic progress, consequently improved livestock productivity. Different respond to the superovulation is important limitation [7], that are caused by multi factors [11-14].

Previous studies have stated that the possible factors affecting in-vivo embryo production such as low fertility rate [15], low viable embryos [15,16], and high rate of unfertilized oocytes [12,16]. Likewise, the quality of embryos is influenced by the heat stress of the donors [17]. On the other hand, several studies have indicated that ET can be applied in dairy cows to increase the fertility rate in comparison to AI, especially in summer months [18-20]. In addition, involving ET in repeat breeders as recipients have shown satisfying results [21,22].

As the results of the application of reproductive technology especially superovulation in Bali cows, that the responses were satisfied, although the number of follicles was still lower than expected, however, it has a possibility when using well-selected Bali cows for donors might be resulted in a high number of development follicles as well as oocytes produced.

4. Conclusions
In conclusion, although the number of oocytes produced in Bali cows after doing superovulation was still lower than expected, however, it is satisfied enough that Bali cows are responded to superovulation.

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