Effectiveness of estrous synchronization using prostaglandin (PGF2α) in Bali cows

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Abstract. The objective of this study was to know the effect of estrous synchronization using prostaglandin (PGF2α) hormone injection in producing the percentage of estrus in Bali cows. Palpation of rectal examination on 70 Bali cows was carried out using plastic gloves to select the cows. The results were 44 Bali cows in pregnant condition, follicular phase, and anestrus. There were 26 Bali cows in the luteal phase, used in this study. All Bali cows in the luteal phase were injected with prostaglandin hormone intramuscularly followed by estrus detection from one day after injection. The parameters measured were the number of Bali cows that become estrus and pregnant, and signs of estrus that expressed. The results of this study showed that the injection of prostaglandin (PGF2α) effectively produced a high response of estrous of Bali cows and the pregnancy rate was accounting for 77%. As conclusions, synchronization of estrus in Bali cows using prostaglandin (PGF2α) is effective in producing a 100% estrus response, the luteal phase in cows was a major success for synchronizing estrus and pregnancy rate was relatively high at first AI.

1. Introduction
Indonesia Ministry of Agriculture predicts that Indonesian meat production will not be able to meet the national needs, whereas demand for beef in the country increase year by year. On this basis, the demand for beef was only met by 60.9% of the national beef production. There are many government programs that aimed in improving the quality and quantity of beef cattle that have been carried out to achieve the need for national beef. One of the national programs currently being launched is the UPSUS SIWAB (Special efforts for cows to become pregnant) which aims to increase the cattle population through artificial insemination (AI) technology.

According to the UPSUS SIWAB data of the Ministry of Agriculture, the achievement of pregnancy rate was 54.7% and calving rate was 36.4% in South Sulawesi Province for the period of January to July 2018. These data show that the achievement of the AI technology was still low whereas one of the possible factors was the cows not in estrus or difficulty to find cows in estrus in the field. The other possible problem was a variation in the estrus time in the cows’ population in the field while the number of inseminators was relatively low. Therefore, one of the programs that might be applied is to synchronize the estrus of the cows.

To solve the problem, in applying artificial insemination in order to optimize the successful rate, an estrous synchronization technique has been developed. Synchronization of estrus is a way to standardize
the mating schedule over a period of time and can be predicted in a group of animals [1]. Synchronization of estrus and ovulation in cows often uses a combination of two or three hormones. A high rate of estrous score indicates good quality of treatment. The clearer the appearance of estrus, the more precise the identification of desire and the more precise the implementation of AI [2].

In synchronizing estrus, one must be known is the activity of the ovaries during the estrus cycle so that it is easier to shorten the cycle. It is hoped that the synchronization of estrus with the injection of the hormone prostaglandin (PGF2α) will increase the success rate of AI. The impacts that occur with the synchronization of estrus and ovulation include earlier calving in the calving season, reduction of dystocia, use of high-quality bulls, and increased weaning weight of the calves. With this synchronization, it facilitates maintenance of various management such as estrus, mating, calving, and weaning of calves. The use of estrous synchronization techniques will increase the efficiency of production and reproduction of the herd, as well as optimize the implementation of AI and increase the fertility of the group [3].

Field evidence shows that the estrus phases in Bali cattle are different (anestrous, follicular, and luteal phases). To produce an appropriate time for AI, it is necessary to apply estrous synchronization in the herd. Therefore, based on the above review, it is necessary to conduct a study on the effectiveness of estrus synchronization with the injection of the hormone prostaglandin (PGF2α). It is suspected that the synchronization of estrus at different ovarian activities using a single prostaglandin in Bali cattle results in a different percentage of estrus. This study aimed to determine the effectiveness of estrous synchronization using prostaglandin (PGF) injection to the number of cows coming into estrus. The advantage of this study is to provide new information in the development of estrous synchronization using the injection of prostaglandin hormone (PGF2α) to produce the percentage of cows in estrus especially in Bali cattle.

2. Materials and methods

2.1. Farm management and Bali cattle
This study was conducted at the Mawi Breeding Center (MBC) Faculty of Animal Science, Hasanuddin University that is located in Enrekang Regency, South Sulawesi. MBC is a small ranch farm with a herd size of greater than 200 Bali cattle. The environment in this ranch is a tropical condition with two main seasons; hot and rainy seasons. Most of the land is cover with natural grasses. All cattle are raised extensively in the paddock during the day-time and in the housing barn during evening-time. During day-time, the cattle are mostly grazing in the paddock with the availability of natural grass and sometimes fed with concentrate in the barn during evening-time.

Basically, mating management is conducted naturally, whereas bulls are involved in the herd. Cows or heifers in estrus are usually mated by the available bulls in the field without any recording. Pregnant cows or heifers are usually calved in the paddock field without any assistance from the staff. The Bali cows used in the present study were raised in the farms equipped with paddock and pens, similar management used in the herd.

2.2. Procedure and parameter of the study
A total of 70 Bali cattle (cows and heifers) was examined for their reproductive status for selection. The examination was conducted by palpation per rectum in order to explore whether the Bali cattle were pregnant or not, the presence of corpus luteum for un-pregnant cattle, as well as possibility for reproductive organ disorder [4]. After selection, there were 26 un-pregnant Bali cattle in the luteal phase that were subsequently noted for treatment and as the main materials of this study. The other materials used were plastic gloves, syringe 5 mL and needle 18 G, hormone prostaglandin (PGF2α).

The selected Bali cows have received an intramuscular injection of prostaglandin (PGF2α). Within three days after PGF2α injection, estrus of each Bali cow was observed visually. The Bali cows in which showing estrus after treatment were then inseminated by a technician using frozen-thaw semen of Bali. Parameters measured in the study were the number of cattle coming into estrus, estrous signs, and
pregnancy rate.

2.3. Study design and data analysis
This study was designed to know the effectiveness of using prostaglandin (PGF2α) in Bali cows especially in the luteal phase. All data were calculated and presented as mean and percentage and tabulated in the Excel program for Windows. Differences in mean and percentage of number of cows coming into estrus, estrous signs, and pregnancy rate were descriptively described.

3. Results and discussion
3.1. Reproductive status of the cows before estrous synchronization
Before conducting estrous synchronization, all Bali cows were subjected to reproductive genital organs examination especially their ovarian activity. The examination was to describe the reproductive status of the cows before estrous synchronization in order to effectively synchronize using PGF2α alone. The results of the reproductive genital organs examination in Bali cows are shown in Table 1.

| Status                  | Number of Bali cows | Percentage (%) |
|-------------------------|----------------------|----------------|
| Pregnant                | 24                   | 35             |
| Anestrus (inactive ovary)| 10                   | 14             |
| Luteal phase            | 32                   | 46             |
| Follicular phase        | 3                    | 4              |
| Abortion                | 1                    | 1              |
| Total                   | 70                   | 100            |

In the present study, out of 70 Bali cows examined, 24 (35%) cows were pregnant and the remaining 65% cows were not pregnant. Table 1 shows that there were 32 (46%) cows in the luteal phase, whereas these in luteal phase cows were the target for estrous synchronization using single prostaglandin (PGF2α) injection. Knowing the phase in the estrus cycle of cows before estrous synchronization is very important in estimating the likelihood of successful treatment [5]. The luteal phase in cows is suitable for synchronizing estrus with the injection of the prostaglandin hormone (PGF2α) because, in the luteal phase, the corpus luteum (CL) actively produces the progesterone hormone which inhibits estrus. By injecting the PGF2α hormone which will lyse the CL so that the progesterone hormone decreases [6]. A decrease in the level of the hormone progesterone results in the loss of negative barriers to the hypothalamus and anterior pituitary so that the production of FSH and LH hormones increases for the development of follicles that produce the hormone estrogen resulting in cows showing estrus [7].

3.2. Response of Bali cows after estrous synchronization
Out of 26 Bali cows synchronized using single prostaglandin (PGF2α), all of them showed estrous with various estrous signs (figure 1). After estrus detection, all Bali cows were artificially inseminated using frozen-thawed semen by the technician. Out of 26 cows that had been inseminated, 77% of them become pregnant at first insemination.
A study of Indira [8] using Bali cows stated that synchronization of estrus with the PGF2α caused a 100% response to estrus, symptoms and normal sexual behavior, normal estrous cycles, and increased estrogen levels during estrus. Hafizuddin et al. [9] stated that lactating beef cows that were administered intramuscular PGF2α caused estrus by 87%. According to Sudarmaji and Gunawan [10], the synchronization of estrus in Bali and PO cows by using the PGF2α injection method twice caused all cows to experience estrus. This may be due to the possibility that all cows were in the luteal phase and having an active CL, so the prostaglandin hormone (PGF2α) plays a role in the regression of the CL formed. This CL regression causes progesterone levels to decrease and results in the loss of inhibition against the gonadotropin hormone which is then followed by growth and maturation of follicles, arising in estrus and ovulation. As stated by Marietta, et al. [11] that the use of the PGF2α hormone for the estrous synchronization program is only effective if the cows have CL. Evidence suggests that prostaglandins play a role in the ovulation process in cattle with corpus luteum regression. Estrous synchronization is one way to increase the success of AI.

Synchronization of estrus with the injection of PGF2α in Bali cattle has the same function as the process of PGF2α secretion by the uterine wall, that aim of restoring the estrus cycle to the follicular phase by stopping the production of the progesterone. PGF2α administration for estrus control can only be done when the corpus luteum has been formed [12]. The results of this study were in line with the study of Millar, et al. [13] which stated that not all cows in estrus can show all the same symptoms of estrous. The time difference between injection of PGF2α and the onset of estrus depends on the phase of the follicular wave at the time of injection of PGF2α [14]. Although the result of synchronization in estrus causes cows to be in estrus, the pregnancy rate only reaches 77% in the present study.

The success rate of AI is strongly influenced by four factors that are interrelated and cannot be separated from one another; the selection of cows to be inseminated, semen quality, the accuracy of estrus detection by the farmers, and inseminator skills. In this case, the inseminator and the farmers are the spearheads of the implementation of AI as well as the parties responsible for the success or failure of the AI program in the field. The success of AI can be influenced by several factors, including the skills of the inseminator in determining the timing and carrying out of the AI procedure, semen quality,
and environmental factors. Inseminator skills in observing signs of estrus, handling frozen semen, etc. will determine the success of AI.

3.3. Estrus signs of Bali cows after estrous synchronization

It can be seen in table 3, that the cows showing signs of estrus after being synchronized using the PGF2α hormone, consists of 77% showed vaginal swelling and 7% had vaginal mucus successfully conceived after artificial insemination (AI). The cows that did not become pregnant showed fewer signs of estrus, i.e., 23% showed vaginal swelling and 7% had vaginal mucus. Kune and Najamudin [15] stated that the difference in the quality of estrus in cows can be caused by individual factors related to hormonal conditions, especially the condition of the estrogen in stimulating sexual activity. Frandson, et al. [16] also stated that estradiol stimulates swelling and reddish the external genitals, and an increase in vaginal secretions of mucus that comes out on the vulva. Injection with PGF2α causes luteolysis of the corpus luteum (CL) in the ovaries and causes the cows to express estrous caused by a decrease in the concentration of progesterone [17].

| Sign of estrus          | Number of Bali cows (%) | Pregnant | Not-pregnant | Total |
|-------------------------|--------------------------|----------|--------------|-------|
| Swelling of vulva       | 20 (77)                  | 6 (23)   | 26           |
| Slightly                | 10 (50)                  | 4 (75)   | 14 (54)      |
| Clear                   | 5 (25)                   | 0 (0)    | 5 (19)       |
| No swelling             | 5 (25)                   | 2 (25)   | 7 (27)       |
| Mucus discharge         | 20 (77)                  | 6 (23)   | 26           |
| Clear                   | 0 (0)                    | 1 (13)   | 1 (4)        |
| Not-clear               | 1 (5)                    | 1 (13)   | 2 (7)        |
| Containing pus          | 0 (0)                    | 0 (0)    | 0 (0)        |
| Slightly                | 1 (5)                    | 0 (0)    | 1 (4)        |
| No mucus                | 18 (90)                  | 4 (75)   | 22 (85)      |

The right time in the implementation of the AI is a major factor that must be considered. At the time of AI cows must be in estrus because at that time the cervix is in an open position [18]. Normally cows in estrus will secrete mucus from the vulva, which is clear, pure, and odorless [19]. The amount and consistency of mucus will change depending on the phase of the estrus cycle and change depending on the variation of hormonal levels. During the estrus cycle, changes in the physical characteristics of cervical mucus can be used to indicate the optimal time to perform AI [20]. Holstein cows with thin and clear mucus have a pregnancy rate of 65% and cows with thick mucus have a pregnancy rate of 40% [21]. Observation of mucus is done by looking at the consistency and amount of mucus that comes out of the vulva before AI with the assessment criteria including 1) thick mucus when it comes out hanging unbroken, the diameter of the mucus is wider, has a high viscosity, and is transparent; 2) the mucus is thin when it comes out hanging broken, small diameter, low viscosity, and transparent; and 3) the mucus does not come out of the vulva, it is considered that the mucus production has stopped.

The results showed that 7% of the cervical mucus was obtained in pregnant and non-pregnant cows. This is due to the presence or absence of a functional corpus luteum that is formed in the acceptor's ovary. The function of PGF2α is to regress and lyse the corpus luteum, with lysis of the corpus luteum the new estrous cycle will start again. The function of hormones works perfectly on target organs if these hormones reach the target organs at the right time and number of doses [22]. The characteristics of cervical mucus play an important role in the success of conception, the appearance of cervical mucus can be used as a tool to detect estrus to determine the right time of insemination in order to get an increase in the percentage of pregnancy [21].

The difference in the quality of estrus that appears is indicated by the difference in the ability of the maximum secretion of sexual hormones for each individual cow. The higher the estrogen hormone
produced, the higher the quality of estrus that will appear. Tsiliganni et al. [20] stated that cervical mucus is produced by secretory cells found in the endocervix, the quality and quantity of cervical mucus are strongly influenced by the condition of hormones secreted during estrus. Hafizuddin et al. [9] support that the circulation of hormones in the body greatly affects the process of follicle growth, ovulation, and the formation of CL. Kune and Najamudin [15] also stated that differences in the quality of estrus in cows can be caused by individual factors related to hormonal conditions, especially the condition of the hormone estrogen in stimulating sexual activity.

During the estrus cycle, there are three waves of follicles. In the first wave of follicles, there are no ovulated follicles, only a dominant follicle is formed. The first follicular wave occurs on day 1 to day 7, while the second follicular wave occurs on day 9 to day 15, and the third follicular wave occurs on day 16 to day 21 leading to estrus during one cycle of estrous. In the third wave of follicles, there is a predominantly ovulating follicle at the end of the estrus cycle. Associated with the luteal phase there is an increase in the hormone progesterone but low gonadotropin and estrogen hormones. Endocrinological imbalances in heifers can be detected from several reproductive disorders such as cystic follicles, silent heat, and early embryonic death. Wahyudi, et al. [23] stated that cystic follicles are follicles that are unable to ovulate because the LH hormone does not reach its peak but is able to cause signs of estrus.

Injection with prostaglandins causes luteolysis corpus luteum (CL) in the ovaries and causes the cow to express estrus due to a decrease in the concentration of progesterone [17]. The PGF2α hormone injected into the cow will generate heat 48-72 hours after PGF2α administration [2]. The time difference between injection of PGF2α and the onset of estrus depends on the phase of the follicular wave at the time of injection of PGF2α [14]. PGF2α administration for estrous control can only be done once the corpus luteum has formed. Therefore, injecting a single dose of PGF2α to synchronize estrus will not guarantee that all animals can be in estrus at once. The PGF2α hormone is transported through the blood circulation so that it acts on target tissues that are far from where it is produced [12].

4. Conclusions
Based on the results and discussion, it can be concluded that: 1) synchronization of estrus in Bali cows using prostaglandin (PGF2α) is effective in producing a 100% estrus response, 2) the luteal phase in cows is a major success for synchronizing estrus, and 3) pregnancy rate was relatively high (77%) at first AI.

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