Superovulatory responses using pregnant mare serum gonadotropin hormone in Murrah buffalo cows

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Abstract. Superovulation plays an important role in embryo transfer program. A preliminary study carried out in the Research Institute for Animal Production evaluated superovulatory responses in Murrah buffalo cows using pregnant mare serum gonadotropin (PMSG) hormone. The three buffalo cows were estrus synchronized using 5 ml prostaglandin (PGF) twice, with an interval of 11 days. PMSG was injected intra-muscularly 3000 IU on day-10 after estrus. Prostaglandins were administered 48 hours after PMSG injection. Fixed-Time artificial insemination (FTAI) was carried out at 72 hours after the last PGF treatment. Administration of hCG 2 ml/head was given at the time of FTAI. A non-surgery flushing was performed on day 6 after FTAI. Parameters observed using ultrasonography (USG) were diameter of follicle (DFL), total follicle (TFL), and number embryos (NE). Data were analyzed descriptively. The mean of DFL before PMSG treatment was 8.2 mm and after PMSG treatment was 12.5 mm. The mean of TFL before PMSG treatment was 7.7 and after PMSG treatment was 12.5 mm. The mean of NE before PMSG treatment was 8.2 and after PMSG treatment was 12.5. The NE obtained was one degenerative embryo. Superovulation using PMSG increased TCL and DFL. It can be concluded that the Murrah buffalo cows superovulated by PMSG showed a good response but no transferable embryo was found.

Keywords: Superovulation, PMSG, USG, follicle, Murrah buffalo cows

1. Introduction
Buffaloes play an important role in the agricultural economy and food security for farmers, including the Murrah buffaloes as a dairy buffalo type producing high and good quality of milk [1]. In the last decade, the Indonesian buffalo population, especially Murrah, has declined significantly because of inbreeding depression occurred due to scarcity of bulls for mating and no mating direction [2, 3] thereby, lowering their production performance [4].

Genetic improvement can be enhanced fast through applied reproductive technologies such as artificial insemination (AI), embryo transfer (ET) and transgenic [5]. AI has been practiced particularly in some buffalo breeding regions resulting in higher performance and farmer preferences [2]. However, the ET program in buffaloes has not been done yet, especially in Murrah.

Multiple ovulation and Embryo transfer technique (MOET) has proven to be a satisfactory tool for faster multiplication of the desired genetic resources used for cattle and buffalo. The use of superovulation followed by fixed time artificial insemination (FTAI) has demonstrated an efficient technique that generates a large number of embryos per donor in buffalo [5].

For many years hormones such as Pregnant Mare Serum Gonadotropin (PMSG) or Follicle Stimulating Hormone (FSH) have been used for the treatment of superovulation in buffaloes [6]. The advantage of using PMSG for superovulation is its availability in large quantities for a low cost. PMSG
can be also administered, as a single dose instead of the multiple injections usually required when using pituitary preparations [7, 8].

The superovulatory effects of PMSG and FSH have been used to increase ovulation rates in buffaloes and have been applied in conjunction with progestagen and/or prostaglandin F2α treatments to regulate the estrus cycle [8]. Many studies reported the different responses of these method combinations were influenced by genetic and non-genetic factors both internals and externals [5, 9, 10].

A preliminary study was conducted to evaluate superovulatory responses using PMSG in Murrah buffalo cows. This information can be used as recommendation for the development of embryo production in buffaloes.

2. Materials and methods

The study was conducted at Research Institute for Animal Production (RIAP) Ciawi, located at 250-350 m above sea level. The utilization of the treated animals in this study has been ethically approved by the Animal Welfare Committee of Indonesian Agency Research and Development, number: Balitbangtan/Balitnak/Rm/02/2020.

This study used three Murrah buffalo cows with averaged body condition score of 3 (good). All animals were housed in individual pens measuring 2x3 m and fed king grass of 30 kg/head/day and commercial concentrate. Drinking water was provided ad libitum.

The buffalo heifers were estrus synchronized using 5 ml prostaglandin (PGF) twice intramuscularly, with an interval of 11 days. Estrus occurred at three days after the second PGF injection. Pregnant Mare Serum Gonadotropin (PMSG) was injected intramuscularly of 3000 IU on day-10 after estrus. PGF was injected 48 hours after PMSG treatment. Fixed-time artificial insemination (FTAI) was carried out 72 hours after the last treatment PGF, using two frozen semen straws per insemination. Administration of 2 ml/head hCG was at the time of FTAI. Table 1 showed the treatment schedule.

Embryo were collected using a non-surgically technique on day 6 of FTAI by flushing each uterine horn using a Foley catheter with 500 ml of Phosphate Buffer Saline containing 0.04% Bovine Serum Albumin (BSA). Prior to flushing, the buffalo was confined in a chute and 2 ml of 2% Xylocaine Hydrochloride was injected to prevent straining and defecation.

Embryos were searched and evaluated morphologically using a microscope and graded according to the manual of International Embryo Transfer Society [11]. Ovarian status was monitored by ultrasonography (USG). Ultrasound scanning was performed by using ExaGo veterinary ultrasound machine fitted with a 7,5 MHz, a transrectal scanner. USG examination was performed before (10 days after estrus) and after PMSG treatment (same day as FTAI). The variables observed were diameter of follicle (DFL), total follicle (TFL), and number of embryos (NE). Data were analyzed descriptively.

| Day | Treatment | Dose                        |
|-----|-----------|-----------------------------|
| -14 | Prostaglandin (PGF) I | 5 ml (intramuscular)         |
| -3  | PGF II    | 5 ml                        |
| 0   | Estrus (I) |                             |
| +10 | PMSG      | 3000 IU (intramuscular)     |
| +12 | PGF       | 5 ml                        |
|     | PGF       | 5 ml                        |
| +15 (0) | Estrus (II) |                           |
|     | FTAI      | 2 straws                    |
|     | hCG       | 2 ml (intramuscular)        |
| +21 (+6) | Flushing (PBS) | 500 ml (Foley catheter)    |

3. Results and discussion

This study was a preliminary study of embryo production in buffaloes using PMSG followed by fixed-time AI (FTAI). The availability of Murrah buffalo cows used in this study were limited, however can used as a preliminary information. The response of superovulation treatment in Murrah buffalo cows
are presented in Table 2. This study showed that the response of Murrah buffalo cows to the superovulation method according to [12].

| Treatment     | TFL of ovary | DFL of ovary, mm | Numbers of embryo (NE) |
|---------------|--------------|-------------------|------------------------|
|               | right | left | total | right | left | mean | degenerative (1) |
| Day-1         | 5.6   | 2.1  | 7.7   | 9.3   | 7.1  | 8.2  |                      |
| Day-5         | 8.9   | 7.3  | 16.1  | 13.3  | 11.6 | 12.5 |                      |

The mean of TFL after superovulation treatment was two times higher than before treatment (16.1 vs 7.7) demonstrating the effectiveness of PMSG. TFL of the left ovary showed a lower response to superovulation treatment (7.3 vs 8.9). The mean of DFL after superovulation treatment was much higher than before treatment (8.2 vs 12.5 mm) indicating more a dominant follicle. Left ovary had lower DFL both before and after treatment of PMSG. However, the difference of TFL and DFL between right and left ovary after superovulation treatment was in line with the study using FSH in Murrah and swamp buffalo heifers [13, 14] and the response was similar to [15] using PMSG. However, another study showed that both left and right ovaries responded equally to PMSG superovulation [16].

The right ovary was related to arterial blood supply to the needs of the organ resulting in better response of superovulation [13, 14]. In this study, total numbers of follicle (TFL) and diameter (DFL) were higher compared to [7] who reported 8-10 TFL, with diameter of > 9 mm studied in Egyptian buffalo using PMSG. Another study found the average of DFL size in bovine using PMSG was 10-14 mm and the TFL was 3-5 [8]. The TCL in this present study was closed to the study using FSH for superovulation treatment in buffalo resulting in 12 CL [13, 14].

Comparing TCL and DFL before and after superovulation treatment proved PMSG could increase number of TCL and DFL size. This present study revealed that PMSG can be used for superovulation in Murrah buffaloes and showed good responses even though, no embryo was found in all cows. Several studies showed that embryo recovery in buffalo was less than 1 viable embryo to 2.5-3/donor and lower than bovines [5]. The response of buffalo ovaries to super-stimulatory treatment was less than one third of that reported in cattle.

These results were in line with those reported by earlier studies in cattle and buffalo. A large number of evidences showed that PMSG produced much larger ovaries [15]. Generally, the effect of PMSG is volume doubling compared to those treated with FSH [15, 16] caused by the sialic acid content in PMSG prolongs the half-life of hormone; which results in continued recruitment of follicles after ovulation. These elongated follicles secreted oestradiol in excess of the normal preovulatory concentration leading poor superovulatory responses [8]. However, the increase of non-ovulating follicles affected the low magnitude of the preovulatory LH surge and a low progesterone concentration during the early luteal phase of the superovulatory cycles. This condition lead to a lower embryo recovered.

Multiple ovulation embryo transfer (MOET) typically results in a relatively low recovery of both embryos and unfertilized ova in buffalo [17]. The proportion of responsive buffaloes varies between 20-40% of subjects that produced at least 1 embryo after superovulation [5]. Poor response to superovulation and low embryo recovery are attributed to low primordial follicle pool of 20% (in swamp) to 30% (in riverine) in buffaloes compared to cattle and high rate of follicular atresia [18, 19].

The effect of PMSG treatment on TCL and DFL obtained in this study was higher. However, no correlation was found on number of recovered embryos. The results of this present study were similar to studies using FSH for superovulation in Murrah and swamp buffaloes [13, 14]. Other study revealed there was a negative correlation between the presence of a high number of large follicles (>8 mm) on the day of embryo recovery and the number of recovered embryos [17]. Therefore, the TCL and DFL in this study, could not be a good predictor for the number of transferable embryos collected.

In general, gonadotrophin hormone (PMSG) can be used for superovulation treatment resulting in an increase in diameter of follicle and TCL. However, this present study showed no effect on recovered embryos. The PMSG hormone can increase the diameter of the follicle, but it is not followed by oviduct
changes resulting in failure of embryo recovery. The capability of fimbriae in collecting the ovum causing failure to get embryos that occurred in buffalo [9]. The failure to get embryos from superovulation treatments may be caused by the size of ovary where the diameter of ovary was extremely big and therefore the fimbriae failed to collect the ovulated ovum. Oviduct fimbriae of buffaloes treated with superovulation hormone, failed to capture the ovum [5].

Many factors have been reported to affect ET in buffalo resulting in a very limited numbers of embryos collected [9]. Other factors affected by MOET were genetic, age and parity of donor, body weight, body condition, management, stage of estrous cycle, type of gonadotropin hormone, and season or climate [5, 9, 10]. In the present study, the number of animals used was very limited, however, they had the same body condition, management treatment and parity. Further, the age of heifers were 2.5-3 years old, with body weight of 300-400 kg and averaged body condition score 3 (good). Response to reproductive technologies such as AI and MOET programs were more efficient when body condition score was between 2.5 and 4 on a scale of 1 to 5 [20]. Therefore, the donors used in this study met the requirement.

4. Conclusions
Murrah Buffalo cows can be hormonally superovulated using PMSG resulting in good responses. The total number of follicle and diameter increased with superovulation treatment using PMSG. No embryo recovery was found and it remains challenging.

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