Superovulation response of Peranakan Ongole (PO) and Simmental cows after FSH stimulation in multiple ovulation and embryo transfer program

A F Lubis, A S Satyaningtijas, O P Lubis, W Kurniati and A Boediono

1Department of Anatomy Physiology and Pharmacology, Faculty of Veterinary Medicine, IPB University, Bogor, Indonesia
2Indonesian Livestock Embryo Center Cipelang, Bogor, Indonesia

Corresponding author: ab@apps.ipb.ac.id

Abstract. Multiple ovulation and embryo transfer (MOET) is a reproductive technology to increase the livestock population in a short period. The success of ovary stimulation programs is influenced by an individual's response to follicle-stimulating hormone (FSH) stimulation. In this study embryo production was carried out on local cows represented by Peranakan Ongole (PO) cows and exotic cows represented by Simmental cows. FSH stimulation was performed on 10 PO cows and 10 Simmental cows. On Day-1 Cue Mate® (progesterone) was inserted intravaginally. FSH injection (400 mg) was carried out intramuscularly from D-10 at reduced dose with 12 hours intervals (Day-10: 100 mg; Day-11: 60 mg; Day-12: 40 mg per injection). On Day-12, prostaglandin (PGF2α) was injected and Cue Mate® was removed. Artificial insemination (AI) was performed 48 hours after PGF2α injection for 3 times at 12 hours intervals. The embryo was collected 6 days after the last AI (Day-21). Superovulation response was detected on 70% of PO cows and 90% of Simmental cows. The average number of transferable embryos in Simmental cows (9.11±7.27) was higher than PO cows (7.86±7.78). This research shows that Simmental cows are more responsive to FSH stimulation, and can produce more transferable embryos than PO cows.

1. Introduction
Multiple ovulation and embryo transfer (MOET) is a reproductive technology that aims to increase the livestock population in a short period. The purpose of ovary stimulation on the MOET program is to optimize the potential of oocytes from donor cows. During the stimulation program, gonadotropins must be available for a long time (at least 72 hours) for follicular growth and oocyte maturation until they are ready to be ovulated [1]. Ovary stimulation in cattle requires the administration of gonadotropin hormone that contains or has a similar effect to the way follicle-stimulating hormone (FSH) works [2]. FSH stimulates the growth of follicles in the ovaries. The development of efficient ovary stimulation protocols has been carried out to reduce production costs and increase the number of transferable embryos. One of the most common stimulation protocols is injecting a decreased dose of FSH for 3 or 4 days. Treatments with multiple FSH injections (six equivalent or decreasing doses) are more efficient to increase the number of aspirated follicles when compared to a single bolus FSH treatment [3]. The success of ovary stimulation programs is influenced by an individual's response to FSH stimulation.
Differences in stimulation response to FSH between individuals can affect the quantity and quality of the embryo.

Indonesia has several local breed cows that have potential to be developed as cattle breeds, one of them is the Peranakan Ongole (PO) cow. PO cows are generally designated as working cows and beef cattle. PO cows have the advantage of being more tolerant of tropical environments with hot temperatures and high humidity, as well as limited feed. In addition to local cattle, exotic breed cows have also been widely cultivated in Indonesia. One of the most common exotic breed cows in Indonesia is the Simmental breed. This study was conducted to analyze the superovulation response and the embryo quality to ovary stimulation with FSH. Embryo production was carried out on local breeds represented by Peranakan Ongole (PO) cows and exotic breeds represented by Simmental cows.

2. Materials and methods

2.1. Experimental animal and groups

Embryo production was carried out on 10 PO cows and 10 Simmental cows that already passed the donor selection according to the existing protocol on Indonesian Livestock Embryo Center. Diet was provided as 45-50 kg of grass per head per day and 4-5 kg of commercial diet per head per day. All the donor cows got the same stimulation protocol.

2.2. Estrus synchronization and ovary stimulation

Estrus synchronization was carried out by insertion of Cue Mate® (progesterone) intravaginally on Day-1. Ovary stimulation with FSH was started on Day-10 until Day-12. FSH (Folltropin-V®) injection (400 mg) was carried out intramuscularly on all the donor cows at a decreased dose for 6 times with 12 hours intervals (Day-10: 100 mg, 100 mg; Day-11: 60 mg, 60 mg; Day-12: 40 mg, 40 mg). Luteolysis was induced by injecting PGF2α (Lutalyse®) at Day-12. Cue Mate® removal was carried out on the day-12 in the morning. Artificial insemination (AI) was done at ± 48 hours after PGF2α injection. AI was done 3 times at 12 hours intervals.

2.3. Embryo collection and evaluation

Embryo collection was done by flushing non-surgically on Day-21 or 6 days after the last AI treatment. The donor cows that have more than 2 corpus luteum (CL) were flushed. Flushing was carried out using a two-way Folley catheter. Before flushing, donor cows were given epidural anesthesia using lidocaine (0.22-0.5 mg/kg). Ringer lactate (RL) supplemented with 1 to 2% (v/v) serum, 200,000 IU/L penicillin, and 0.1 g/L streptomycin, was used as a flushing medium. The flushing medium was warmed until the temperature reached 38.5°C. One donor cow needs 3 bottles (@500 mL) of medium flushing, each bottle used to flush right cornua, left cornua, and corpus uteri. The liquid from flushing was filtered to facilitate embryo collection. The collected embryo from each donor was pooled into a petri dish. Evaluation and classification of embryos were carried out morphologically using a microscope. The obtained embryos are classified based on the phase or development stage and the quality of the embryo.

3. Results and discussion

3.1. Stimulation response

Stimulation response can be determined by the number of the obtained embryos. Cow with 3 or more than 3 embryos means that the cow is classified as a good response to the stimulation. Otherwise, the cow with less than 3 embryos is classified as a poor response to the stimulation. The stimulation response based on the total number of embryos obtained did not show any significant difference between PO cows and Simmental cows (P>0.05). But as an individual, 7 out of 10 PO cows (70%) and 9 out of 10 Simmental cows (90%) showed good stimulation responses (Table 1). The average amount of corpus luteum (CL) was higher on Simmental cows than PO cows. Likewise, the average number of embryos per individual showed higher yields in Simmental cows (12.80±9.26) compared to PO cows.
This result shows that Simmental cows have better response to FSH stimulation than PO cows. In previous studies on non-lactating Simmental cows, the average count of total CL and embryo from cows that were stimulated with FSH 400 mg were 12.82 ± 6.94 and 12.50 ± 6.79 [4].

Table 1. Stimulation response in PO and Simmental cows following the FSH injection

|                | Embryo > 3 | Embryo <3 | P-value |
|----------------|------------|-----------|---------|
| PO (n=10)      | 7          | 3         | 8.90±11.75 | 8.90±11.75 | P>0.05 |
| Simmental (n=10)| 9         | 1         | 13.20±9.65 | 12.80±9.26 | P>0.05 |

Various factors have been reported to influence the stimulation response in cows. These include intrinsic factors that are related to the physiological status of the animal such as age, genetic differences, and ovarian status at the time of stimulation. Other factors such as season, nutrition and hormone preparations can be considered as extrinsic factors [5]. Differences in stimulation response can be caused by the differences in follicular waves on each cow. The majority of bovine estrous cycles (i.e., >95%) are composed of either two or three follicular waves in the 21-day-estrous cycle [6]. Previous study on PO cows, from 9 cows, 6 cows (66%) showed 3-follicular wave pattern, 3 cows (33%) showed 4-follicular wave pattern and there was no 2-wave pattern [7]. The differences of follicular waves can affect the response of each individual to the given hormone stimulation. Another study said that the wave amount in 1 cycle was not affected by cow breed [6], but there was a 3-wave pattern found in poor nutrition and heat-stressed cattle [8]. An ovary stimulation protocol may have a good result on cows with two follicular waves, but can also not be suitable for cows with three follicular waves, vice versa. This is related to the timing of hormone injections during stimulation treatment.

The outcome of ovary follicular stimulation protocols is influenced by the number of follicles that are stimulated to grow and the number induced to ovulate. Follicular responses to stimulation are optimal when gonadotropin treatment is initiated at the emergence of a new follicular wave. In this situation, follicles are relatively small at the commencement of stimulation, therefore, require additional time to complete maturation before exposure to an LH surge [9]. LH surge is important because it initiates the beginning of ovulation and triggers ovulation about 24 to 36 hours later. The control of the LH surge in ovary stimulation protocols has been studied. The main strategy has been to postpone the LH surge with prostaglandin (PGF) treatment, thereby, allowing more follicles to develop and acquire the capacity to ovulate [10].

3.2. Quality of embryo

The quality of the embryo evaluated by phase of embryo development and grade of the embryo refers to assessment standards by the International Embryo Transfer Society (IETS). Grading was done by looking at the shape of the embryo, blastomere uniformity, percentage of viable cells, and the condition of the zona pellucida. Grade 1: the shape of an embryo is symmetrical; blastomeres are uniform in size, color, and density; at least 85% of cellular material is intact and viable; zona pellucida is round and smooth. Grade 2: slightly irregular shape, at least 50% of cellular material is intact and viable. Grade 3: predominantly irregular in shape, have only 25% intact and viable cellular material. Grade 4: degenerated or non-viable embryo (DG); unfertilized oocytes (UF). Transferable embryos are embryos with grades 1, 2, and 3. Embryos with grade 4 are non-transferable embryos.

Embryo collection was done six days after the last AI, to get embryos at least on the morula phase, that are suitable for embryo transfer. Embryos obtained in this study varied from morula, early blastocyst, blastocyst, expanded blastocyst, hatched blastocyst, to expanding hatched blastocyst phases. The highest distribution of transferable embryos was in early blastocyst (16.8%), blastocyst (48.9%), and expanded blastocyst (27.7%).

Total transferable embryos were not significantly different between PO and Simmental cows (P>0.05). The average number of transferable embryos from individuals that showed a stimulation response showed a higher value in Simmental cows (9.11±7.27) than PO cows (7.86±7.78). The percentage of non-transferable embryos in PO cows (37.08%) was higher than in Simmental cows (36%)
(Tabel 2). The number of DG embryos was higher in Simmental cows (2.70±3.47) than PO cows (1.0±1.25). Embryo damage can be caused by disturbances during embryonic development. A previous study showed that the presence of a CL at the start of stimulation treatment exerted a protective effect on embryonic viability and decreased the degeneration of embryos [11].

**Table 2. Quality of embryo in PO and Simmental cows following the FSH stimulation**

|                      | Transferable embryo | Non-transferable embryo |
|----------------------|---------------------|-------------------------|
|                      | %                   | DG                      | UF                      |
| PO                   | 7.86±7.78           | 37.08                   | 1.00±1.25               | 2.30±4.32               |
| Simmental            | 9.11±7.27           | 36.00                   | 2.70±3.47               | 1.90±1.52               |

The number of UF was higher in PO cows (2.30±4.32) than in Simmental cows (1.90±1.52). Another study reported that superstimulation could affect fertilization rate and the viability of the embryos by creating a negative effect on oocyte and granulosa cell maturation [12]. Fertilization rate in cows that are undergoing superovulation may be lower (50–70% vs 90%) than that of normal cyclic cows [13]. High number of UF indicates that the fertilization is not optimal. Failure to detect estrus and incorrect timing of insemination is often a contributing factor to poor fertilization and low embryo recovery in MOET programs. The success of fertilization can also be influenced by the quality of the semen used, the uterine environment that does not support fertilization, and the imperfect insemination technique. In conclusion, ovary stimulation with reduced dose of FSH is suitable for the MOET program especially in exotic breed cows. This research shows that Simmental cows are more responsive to FSH stimulation, and can produce more transferable embryos than PO cows. We suggest further studies using the different ovary stimulation protocols for efficient superovulation in local breed cows.

**Acknowledgments**

We thank Livestock Embryo Center Cipelang, East Java, Indonesia for all the materials and place that was used in the experiment.

**References**

[1] Bo G A and Mapleton F J 2020 *Theriogenol*. 150 353–9
[2] Karunakaran M, Veerapandian C, Subramanian A, Kathiresan D, Dhali A, Bhatt B P, Manokaran S 2009 *Indian J. Anim. Repro*. 30 33–7
[3] Chaubal S A, Ferre L B, Molina J A, Faber D C, Bols P E J, Rezamand P, Tian X and Yang X 2006 *Theriogenol*. 67 719–28
[4] Erdem H, Alkan H, Karasahin T, Dursun S, Satilmis F and Guler M 2020 *Turk. J. Anim. Sci*. 44 1250–59
[5] Kafi M and McGowan M R 1997 *Anim. Reprod. Sci*. 48 137–57
[6] Adams G P, Jaiswal R, Singh J and Malhi P 2008 *Theriogenol*. 69 72–80
[7] Imron M, Supriatna I, Amrozi and Setiadi M A 2016 *JITV*. 21 26–33
[8] Bo G A, Baruselli P S, Martinez M F 2003 *Anim. Reprod. Sci*. 78 307–26
[9] D’Occhio M J, Jillella D and Lindsey B R 1999 *Theriogenol*. 51 9–35
[10] Bo G A, Baruselli P S, Chesta P M and Martins C M 2006 *Theriogenol*. 65 89–101
[11] Vegia-Lopez A, Gonzalez-Bulnes A, Gracia-Gracia R M, Dominguez V and Cocero M J 2005 *Theriogenol*. 63 1973–83
[12] Hyttel P, Callesen H, Greve T and Schmidt M 1991 *Theriogenol*. 35 91–108
[13] Sartori R, Bastos M R and Wiltbank M C 2010 *RFD*. 22 151–8