Jurnal Agripet
p-ISSN: **1411-4623** | e-ISSN: **2460-4534**
available online at http://jurnal.usk.ac.id/agripet

Vol 24 (2): 168-173, October 2024
Accredited: 152/E/KPT/2023

Reproductive Performance of Ongole Grade, Simmental, and Limousin Cattle Given Superovulation Treatment

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ABSTRACT. Reproductive technologies have the potential to enhance livestock productivity, with superovulation being one such method aimed at increasing the number of ovulated oocytes per estrous cycle through hormonal stimulation. This study aimed to evaluate the reproductive performance of three cattle breeds—Ongole Grade (OG) (n=20), Simmental (n=18), and Limousin (n=20)—subjected to superovulation treatment. The cattle, aged 2.5 to 4 years with a body condition score of 3.5 to 4, were intensively managed. Superovulation was induced using CIDR (Controlled Internal Drug Release) for synchronization, followed by follicle-stimulating hormone (FSH) administration. Reproductive performance data, including age at first estrus, number of corpora lutea (CL), and embryo production, were collected over three estrous cycles. A quasi-experimental design was employed with breed as the primary treatment factor, and data were analyzed using analysis of variance (ANOVA). The results indicated that breed had a significant effect ($P<0.05$) on the age of first estrus, with OG cattle reaching first estrus at 621.3±93.3 days, Simmental at 522.4±75.5 days, and Limousin at 570.4±88.1 days. However, no significant differences ($P>0.05$) were observed among the breeds in terms of the number of CL, collected embryos, or transferable embryos across the superovulation cycles.

Keywords: cattle breed, embryo, reproduction, superovulation

INTRODUCTION

The growing demand for livestock products, particularly meat, is driven by population growth, rising living standards, and changes in consumer lifestyles. To meet this demand, innovations in livestock reproductive technologies are essential (Imron *et al*., 2016). The application of such technologies can accelerate the enhancement of genetic quality, productivity, and livestock populations for meat production. Reproductive technologies in livestock include a variety of methods, ranging from artificial insemination to the more complex procedure of embryo transfer (ET). Embryo transfer involves implanting embryos from donor cows into the uterus of recipient cows. This technology enhances genetic improvement, increases productivity, and supports livestock population growth. To optimize these programs, it is critical to ensure that female cattle have optimal oocyte production.

Low reproductive efficiency, which typically results in the production of only one ovum per reproductive cycle (Andriani *et al*., 2009), limits reproductive potential. Superovulation technology, which stimulates the production of multiple oocytes, can help unlock the full reproductive potential of livestock. Normal female cattle possess around 140,000 oocytes between the ages of 4 and 6 years, but this number declines to approximately 25,000 by the age of 10 to 14 years. On average, from 60 days after birth to 10–14 years of age, a cow produces 150 to 250 follicles. Superovulation is used to stimulate the growth, development, and maturation of follicles, thereby increasing the number of ovulations in a single estrous cycle (Supriatna, 2018). Naturally, cows produce 6 to 8 calves over their productive lifespan (Imron *et al*., 2016), but superovulation can significantly increase the number of oocytes available for fertilization.

The primary objective of superovulation is to maximize the number of oocytes ovulated in each estrous cycle, thus increasing the probability of multiple fertilized eggs when the cow is mated. This technique can be utilized to produce large numbers of cattle with superior genetic traits in a shorter time frame (Supriatna, 2018; Wahjuningsih *et al*., 2019). The application of superovulation biotechnology holds promise for increasing the population of superior cattle, both male and female, particularly those widely bred in Indonesia. However, differences in donor cattle responses to superovulation serve as an indicator of varying reproductive performance across breeds (Tribulo *et al*., 2012; Imron *et al*., 2016). Understanding these differences in reproductive performance is critical for maximizing the efficacy of superovulation technology in livestock.

**Corresponding author*: syahidpanji@gmail.com
Received: 6 June 2023
Revised: 13 March 2024
Accepted: 5 September 2024
DOI: https://doi.org/10.17969/agripet.v24i2.32335

MATERIALS AND METHODS

This study utilized reproductive performance data from 58 female cattle, aged 2.5 to 3 years, with an average body condition score (BCS) of 3.5 to 4. The cattle included 20 Ongole Crossbreed (OC), 18 Simmental, and 20 Limousin cows. Superovulation was induced using Controlled Internal Drug Releasing (CIDR) synchronization in conjunction with follicle-stimulating hormone (FSH) administration. The secondary data were obtained from embryo production records collected during the first, second, and third estrous cycles from the Embryo Hall Livestock (EHL), Cipelang - Bogor. The data parameters included age at first estrus, number of corpora lutea (CL), number of embryos collected, and the number of embryos deemed suitable for transfer.

The age of first estrus was recorded as the age at which each cow first exhibited signs of estrus, indicating reproductive maturity. The number of corpora lutea, representing the count of mature follicles in a single estrous cycle, was measured through rectal palpation, followed by confirmation using ultrasound. Embryo data consisted of the total number of embryos successfully harvested from cattle that had undergone superovulation treatment. The number of transferable embryos—defined as viable embryos of quality grades A, B, or C—was also recorded for each cow (Jodiansyah *et al*., 2013).

Descriptive data were analyzed using analysis of variance (ANOVA) to determine the effect of cattle breed on reproductive performance following superovulation treatment. The variance analysis model, as described by Gasperz (1995), was used and is presented as follows:

$$Y_{ij} = \mu + \alpha_i + \varepsilon_{ij}$$

where:

Y_{ij} = Reproductive performance data for the i-th genotype and j-th replication
μ = Overall mean
α_i = Effect of the i-th genotype
ε_{ij} = Error term for the i-th genotype and j-th replication
i = i-th genotype (1, 2, 3)
j = j-th replication (1, 2, ..., n)

To further evaluate differences in reproductive performance between breeds, Duncan's multiple range test was applied to compare the mean values.

RESULTS AND DISCUSSION

The number of corpora lutea (CL) was observed through rectal palpation, with confirmation using ultrasonography (USG) to accurately determine the number of CLs produced. Once the number of CLs was confirmed, the donor cows underwent a flushing process to collect the embryos optimally. The results of embryo production from the first, second, and third cycles are presented in Table 1.

Table 1. Data recording embryo production for PO, Simental, Limousin cattle

Indicator	Cow Breed								
	Ongole Crossbreed			Simental			Limousine		
	First	Second	Third	First	Second	Third	First	Second	Third
Corpus Luteum (CL)	8.48	9.82	9.40	8.22	8.17	8.91	7.54	8.24	9.15
Embryo Collected	7.11	8.08	6.15	6.22	6.06	6.42	5.34	6.24	6.85
Transferable Embryo	4.84	5.91	4.31	4.06	3.78	4.35	3.25	3.68	5.13

Age of Puberty

The age of puberty is a critical parameter in assessing reproductive performance. Earlier onset of puberty, indicated by the occurrence of the first estrus, allows for earlier mating, thereby improving reproductive efficiency in livestock. Data on the age of puberty in the studied cattle are presented in Table 2. Based on the data in Table 1, Simmental cattle reached puberty earlier, with an average age of 522.4 ± 75.5 days, while Ongole Crossbreed (OC) cattle exhibited the longest time to puberty. These findings align with Rahmayuni (2020), who reported that the age of puberty in Simmental cattle ranges from 435 to 629 days, depending on height. Similarly, Sutomo *et al*. (2013) stated that Bos taurus breeds tend to reach puberty at an average age of 465 ± 50.3 days. In this study, OC cattle reached puberty at 621.3 ± 93.3 days, which is slightly earlier than the findings of Rohayati and Christi (2017), who reported that OC cattle attain puberty at around 22 months, or approximately 660 days.

Analysis of variance (ANOVA) and Duncan's test were used to compare the age of puberty across the three cattle breeds. The results indicated significant differences ($P<0.05$) in the age of puberty. OC cattle had a significantly longer time to puberty compared to Simmental and Limousin cattle, while there was no significant difference between the two subtropical breeds. Various factors, including genetics, nutrition (Rahmayuni, 2020), environmental temperature, humidity (Rohayati and Christi, 2017), and altitude (Sutomo *et al*., 2013), are known to influence the variation in puberty onset among breeds. The genetic influence, in this case, the breed difference, plays a major role in determining puberty age. Additionally, OC cattle have a slower growth rate compared to Simmental and Limousin, which contributes to their delayed puberty (Adriani *et al*., 2018).

Table 2. Data on age of puberty, CL, embryos collected and suitable for transfer for PO, Simental, Limousin cattle

Indicators	Cow Breed		
	Ongole Crossbreed (OC)	Simental	Limousine
Age of Puberty (days)	621.3±93.3[b]	522.4±75.5[a]	570.4±88.1[a]
Corpus Luteum	9.23±6.0[a]	8.43±4.1[a]	8.3±3.8[a]
Embryo Collected	7.1±4.7[a]	6.2±3.3[a]	6.1±3.1[a]
Transferable Embryo	5.02±3.4[a]	4.06±2.3[a]	4.02±2.3[a]

This study was conducted at the Embryo Hall Livestock (EHL) in Cipelang, Bogor, located at an altitude of approximately 1200 meters above sea level. Environmental factors, such as temperature and humidity, are also thought to contribute to the slower onset of puberty in OC cattle (Rohayati and Christi, 2017). In zebu cattle (Bos indicus), puberty is marked by the first ovulation, which occurs between 6 and 24 months of age, depending on genetic and environmental factors (Rawlings *et al*., 2003). Since all three breeds in this study were provided with the same nutrition, environmental conditions, and disease management, the primary factor responsible for the differences in puberty age is the genetic variation between breeds.

Corpus Luteum Amount

The corpus luteum (CL) is formed from follicles that have ruptured and released ova (Supriatna, 2018). CLs in the donor cows' ovaries were examined on the seventh day following superovulation treatment. The number of CLs was determined through rectal palpation and confirmed using ultrasonography (USG). Data on the number of CLs in the three cattle breeds studied are presented in Table 2.

The results indicated an average of 9.23±6.0 CLs for Ongole Crossbreed (OC) cattle, 8.43±4.1 for Simmental cattle, and 8.3±3.8 for Limousin cattle. These results are somewhat lower than those reported by Imron *et al*. (2016), who found that the number of CLs in OC cows treated with superovulation ranged from 10.66±4.9. Similarly, Jodiansyah *et al*. (2013) reported a range of 9±6.8 CLs for Simmental cattle, while Limanu *et al*. (2018) reported 12.28±5.7 CLs for Limousin cattle. To assess the differences in CL numbers among the three cattle breeds, analysis of variance (ANOVA) and Duncan’s test were conducted. The results showed no significant differences (P>0.05) in the number of CLs across the breeds, indicating that the superovulation response, in terms of CL production, was consistent across the three breeds.

Factors influencing the response to superovulation can be categorized into internal and external factors (Silva *et al*., 2009). Internal factors include genetics (breed and sensitivity to hormones), physiological conditions (age, ovarian health, presence of dominant follicles), nutrition (body condition score and nutrient adequacy), and reproductive organ health. External factors involve the administration of exogenous FSH (balance of FSH and LH), FSH dosage, season, and management practices in the field (Jodiansyah *et al*., 2013). FSH and LH, which are produced by the anterior pituitary gland, are responsible for follicle maturation and ovulation. The administration of exogenous hormones in a superovulation program can stimulate excessive follicular growth and maturation, leading to multiple ovulations. These external factors, particularly hormone administration, mitigate the influence of genetic differences between breeds on the superovulation response. As noted by Hiraizumi *et al*. (2015), the administration of FSH can produce a similarly high superovulation response across different breeds. Additionally, superovulation affects the number of CLs formed, which in turn reflects the number of eggs ovulated (Darlian *et al*., 2022).

Number of Embryo Collected

Embryo collection in superovulated donor cows was conducted between the 7th and 8th day after the first artificial insemination. The number of embryos collected from Ongole Crossbreed (OC), Simmental, and Limousin cattle aligns with Jodiansyah *et al*. (2013), who reported that Simmental cattle produced 6.7±3.06 embryos per superovulation cycle. For OC cattle, embryo production ranged between 9.0±2.5 (Imron *et al*., 2016), while Limousin cattle produced 10.1±5.1 embryos (Liamanu *et al*., 2018). The data obtained in this study were generally lower than those reported in earlier studies. An analysis of variance (ANOVA) revealed no significant differences (P>0.05) in the number of embryos collected among the three breeds, indicating that embryo collection in superovulated OC, Simmental, and Limousin cattle is comparable.

Factors influencing embryo yield in superovulated donor cows include age. Young cows (2-4 years) tend to produce fewer embryos compared to donor cows aged 5-10 years (Maulana, 2019). Wahyudi *et al*. (2013) suggested that fertility improves with age, as older cows tend to have better reproductive performance. Malhi *et al*. (2005) added that optimal cow age and condition increase embryo yields. Both younger and older cows typically produce fewer embryos than cows in their prime reproductive years. The administration of exogenous FSH in superovulation programs also contributes to the uniformity of embryo numbers across the three breeds.

Number of Transferable Embryo

The success of superovulation programs in donor cows is primarily determined by the percentage of embryos suitable for transfer to recipient cows. Transferable embryos are those with proper morphological development, classified into quality grades A, B, or C. Embryos unsuitable for transfer include those with abnormal development, degeneration (DG), or unfertilized (UF) oocytes. The ideal developmental stages for embryo transfer range from compact morula to blastocyst (Jodiansyah *et al*., 2013).

Table 2 shows that the number of transferable embryos in this study is generally higher than that reported by Imron *et al*. (2016), who observed 3.67±3.21 transferable embryos in OC cattle. Jodiansyah *et al*. (2013) reported 2.7±5.81 transferable embryos in Simmental cattle, while Maulana (2019) found 5.0±3.94 transferable embryos in Limousin cattle. ANOVA results revealed no significant differences (P>0.05) in the number of transferable embryos among the breeds studied, indicating similar outcomes for superovulated OC, Simmental, and Limousin cows.

The difference between the number of collected and transferable embryos may result from degenerative embryos and UF oocytes, which are not suitable for transfer. Factors contributing to this discrepancy include insemination techniques, inseminator expertise, nutritional deficiencies, and genetic factors. The nutritional status of donor cows plays a critical role in embryo quality and quantity; cows with adequate nutrition tend to produce higher-quality embryos (Lozano *et al*., 2003). Susilawati *et al*. (2016) noted that low fertilization rates could result from poor semen quality due to freezing, inappropriate insemination timing, and incorrect techniques. Additionally, younger cows tend to produce fewer transferable embryos. Maulana (2019) found that reproductive performance improves with age.

Physiological factors during embryo harvesting can affect the number of transferable embryos. During harvesting, donor cows are in the luteal phase with many CLs formed. These CLs produce high levels of progesterone, which supports embryo viability (Jodiansyah *et al*., 2013). The presence of functional CLs ensures embryo survival and reduces early embryo death (Pranata *et al*., 2016). The hormonal and environmental factors optimized in this study minimized genetic influences, resulting in similar numbers of transferable embryos in OC, Simmental, and Limousin cows after superovulation.

CONCLUSION

The reproductive performance of Ongole Crossbreed (OC), Simmental, and Limousin cattle shows differences in the timing of the first estrus, with Simmental and Limousin cattle reaching first estrus earlier than OC cattle. However, reproductive parameters such as the number of corpus luteum (CL), collected embryos, and transferable embryos are not significantly affected by breed differences. Despite this, OC cattle demonstrated a superior response to superovulation compared to the other breeds.

ACKNOWLEDGEMENT

We extend our gratitude to the members of the Production and Application division, particularly those at the Animal Embryo Center Laboratory in Cipelang-Bogor, for their invaluable cooperation. This research was also made possible through the support and facilitation provided by the Head of EHT Cipelang, Bogor.

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