Embryo Production and Development from Superovulated Donors in Double-Muscled Cattle and Their Crosses

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ABSTRAK

Irma, Rasad SD, Hilmia N, Sumantri C. 2023. Produksi dan perkembangan embrio sapi donor double-muscled dan persilangannya yang disuperovulasi. JITV 28 (3):187-196. DOI:http://dx.doi.org/10.14334/jitv.v28.i3.3148.

Introduksi sapi Belgian Blue ke Indonesia dilakukan untuk meningkatkan keanekaragaman sumber daya genetik ternak. Persilangan Belgian Blue (BB) dengan sapi Peranakan Ongole (PO) dilaksanakan untuk meningkatkan produktivitas sapi lokal. Tujuan penelitian ini yaitu menganalisis respon superovulasi, perkembangan embrio praimplantasi dan kualitas embrio *in vivo* Belgian Blue, Peranakan Ongole dan persilangannya. Sinkronisasi estrus dilakukan secara intravaginal menggunakan progesteron Cue-Mate sebelum inseminasi buatan (IB). Superovulasi menggunakan *Follicle Stimulating Hormone* (FSH) secara intramuskuler dan panen embrio dilakukan secara non-bedah. Penilaian kualitas embrio secara mikroskopis mengacu pada kritera *International Embryo Transfer Society* (IETS). Metode penelitian yang digunakan adalah quasi eksperimental, data dianalisis dengan analisis sidik ragam (analysis of variance). Oosit dan embrio berhasil diperoleh dari semua bangsa donor setelah disuperovulasi. Terdapat perbedaan produksi oosit dan embrio donor Peranakan Ongole dan Belgian Blue (berturut-turut PO dan BB yaitu 11.83±1.91 *vs* 4.86±1.33, $p<0.05$). Terdapat perbedaan *recovery rate* (89.63% *vs* 75.35%) dan tingkat fertilisasi (77.35% *vs* 68.22%) antara PO dengan BB. Tidak terdapat perbedaan antara fase perkembangan dan kualitas embrio, serta proporsi embrio layak transfer antar bangsa donor BB, PO dan persilangannya. Penelitian ini menunjukkan bahwa sapi donor crossbred hasil persilangan Belgian Blue dengan PO memiliki karakteristik produksi, *recovery rate*, tingkat fertilisasi dan embrio degeneratif yang sama dengan bangsa murninya.

Kata Kunci:, Flushing Embrio, *In Vivo*, Pra-implantasi, Sapi, Superovulasi

ABSTRACT

Irma, Rasad SD, Hilmia N, Sumantri C. 2023. Embryo production and development from superovulated donors in double-muscled cattle and their crosses. JITV 28 (3):187-196. DOI:http://dx.doi.org/10.14334/jitv.v28.i3.3148.

Belgian Blue was introduced in Indonesia to increase the biodiversity of livestock genetic resources. Belgian Blue was crossed with Ongole grade to increase the productivity of local cattle. Therefore, this study evaluates reproduction traits, especially the response to superovulation, embryonic development, and quality of Belgian Blue, Ongole grade, and their crosses. Estrous was synchronized with intravaginal progesterone Cue-Mate before artificial insemination (AI). Superovulation was performed with Follicle Stimulating Hormone (FSH) intramuscularly with non-surgical embryo flushing. In addition, embryo quality was assessed microscopically according to the International Embryo Transfer Society (IETS) criteria. The study was performed in a quasi-experimental design, and data were analyzed with an analysis of variance. After superovulation, oocytes/embryos were obtained from all donor breeds. Oocyte and embryo production from Ongole grade and Belgian Blue differed at 11.83±1.91 and 4.86±1.33, respectively, $p<0.05$ (mean ± SEM). In addition, there are differences in recovery rate (89.63% vs. 75.35%) and fertilization rate (77.35% vs. 68.22%) between Ongole grade and Belgian Blue, respectively ($p<0.05$). There is no difference in embryo development quality and proportion of transferable embryos between Ongole grade, Belgian Blue, and their crosses. This study concluded that the cross-bred Belgian Blue x Ongole donor had identical oocyte and embryo production, recovery rate, fertilization rate, and degenerative embryos compared to its purebred.

Keywords: Cattle, Embryo Flushing, *In Vivo*, Pre-implantation, Superovulation

INTRODUCTION

Bovine embryo transfer is essential for reproductive biotechnology to improve female fertility with shorter generation intervals, higher selection intensity, and improved selection accuracy for genetic improvement (Jaton et al. 2016). Approximately 387769 bovine embryos are generated in vivo by superovulation, while more than 1000000 are generated *in vitro* (Viana 2021). In addition, the *in vivo* method results in higher pregnancy success, fewer dead fetuses (Sartori et al. 2016), and high-quality embryos (Marsico et al. 2019).

Variability in individual animal responses to superovulation and the low average number of transferable embryos remain the limiting factors of this technology (Center et al. 2018). Season, physiology, and age differences affect superovulation (Vieira et al. 2014).

Bos taurus and *Bos indicus* exhibit differences in reproductive characteristics. *Bos indicus* has more significant retrieved oocytes due to a greater antral follicle count than Bos taurus (Sartori et al. 2016). They can adapt to heat stress and humidity, rusticity, resistance to endo and ectoparasites (Porto-Neto et al. 2014), poor pasture quality, and a tendency for larger oocytes (de Vasconcelos et al. 2020; de Lacerda et al. 2020; Oliveira et al. 2019; Sales et al. 2015; Guerreiro et al. 2014). However, *Bos taurus* has been subjected to more extensive selection and generally has better productivity, including early maturity (Coffey et al. 2016; Madalena et al. 2015). Therefore, cross-breeding is an alternative to using complementarity and heterosis. Consistent with herd performance improvement, cross-breeding resulted in variation (including reproductive traits) among individuals (Marsico et al. 2021). Jemal et al. (2021) reported that superovulation response was higher in crossbreds (*Bos taurus* x *Bos indicus*).

The Ongole grade is a zebu cattle species widely distributed in the tropical climate of Indonesia. Genetic improvement has been achieved through selection and crossing with other breeds, such as Belgian Blue, a taurine breed recently introduced to improve native livestock diversity. A unique characteristic of this Belgian Blue breed is double muscling due to myostatin mutation. In addition, double-muscled crossbreds are expected to provide better performance production with good adaptability and increase reproduction fertility. Bunning et al. (2019) reported that the heterosis of fertility rate is about 12%. Reproductive traits with low heritability are limited to improvement and can lead to maximum heterosis when crossed (Kirkpatrick 2017).

Donor heritability in embryo production ranges from 0.14-0.19, suggesting that embryo production is influenced by genetics (Jaton et al. 2016). Gomez et al. (2020) found that breeds influence fertility. In addition, Belgian Blue was introduced to improve the performance of local cattle in Indonesia through cross-breeding. A study on the myostatin gene of its crossing with Ongole grade was polymorphic but could not distinguish between normal and double-muscled phenotypes (Jakaria et al. 2021). The crossing with Limousin cattle resulted in better in vitro embryo development and quality than pure Belgian Blue (Residiwati et al., 2020). A previous study showed that embryo production was not different between Belgian Blue, Belgian Blue x Simmental, and Belgian Blue x Holstein (Darlian et al. 2021).

Despite the numerous studies on bovine embryo production, little is known about Belgian Blue embryos derived in vivo from cross-bred animals living in tropical climates. The present study investigated the characteristics of the Belgian Blue x Ongole grade cross-bred and its purebred on reproductive performance as donors in a nucleus breeding program using multiple ovulation embryo transfers (superovulation). The present study aims to determine the effects of subspecies (Bos taurus, Bos indicus, and their crosses) on superovulation response, embryo developmental stage, and quality from Ongole grade, Belgian Blue, and Belgian Blue x Ongole. With this background, the research objectives were (1) to evaluate the superovulation response of Belgian Blue (Bos taurus), Ongole grade (Bos indicus), and their cross (½ *Bos taurus* x ½ *Bos indicus*). (2) Evaluation of the donor breeds' pre-implantation developmental stage and embryo quality.

MATERIALS AND METHODS

Ethical statement

The Ethical Committee, Universitas Padjadjaran, Bandung, West Java, Indonesia, approved the research. The Approval Number is 132/UN6.KEP/EC/2022, registration number 2201050051, dated February 11th, 2022.

Materials

Thirty-three donors consisting of 8 *Bos taurus* (Belgian Blue), 10 *Bos indicus* (Ongole grade), and 15 crosses (½ *Bos taurus* x ½ *Bos indicus*) were used for data collection. From 2017 to 2021, the donor was flushed 22, 35, and 17 times for Belgian Blue, Ongole grade, and crossbreds, respectively. Cross-bred cattle are heifer donors derived from the artificial insemination of Belgian Blue semen with Ongole cows (½ *Bos taurus* and ½ *Bos indicus*). Table 1 shows the donor structure. In addition, 633 oocytes/embryos were obtained from the superovulated donor. This study was conducted at the Livestock Embryo Breeding Centre in Bogor Regency, West Java, Indonesia, at 1240 m above sea level, at 18-22 C and 70-80% relative humidity. All donors were clinically healthy, had a body weight of 475-535 kg, a body condition score of 3.0-3.5, and were not lactating. They were caged in the accessible stall and fed 40-50 kg/day of *Pennisetum purpureum*, 5 kg/day of concentrate, and water ad libitum.

Experimental design

The method used was quasi-experimental with a completely random design. *Bos taurus* (Belgian Blue), *Bos indicus* (Ongole grade), and the cross ½ *Bos taurus*

Table 1. Structure of the number of donors, flushing time, the total number of oocytes/embryos obtained, and physiological condition of cattle

Description	Ongole Grade	Cross-bred	Belgian Blue
Number of donor cattle (n)	10	15	8
Body Condition Score (BCS)	3	3	3.5
Age at superovulation (years)	3-5	3-4	2-4
Number of flushing (times)	35	17	22
Physiological condition of the animal	Not lactating	Not lactating	Not lactating

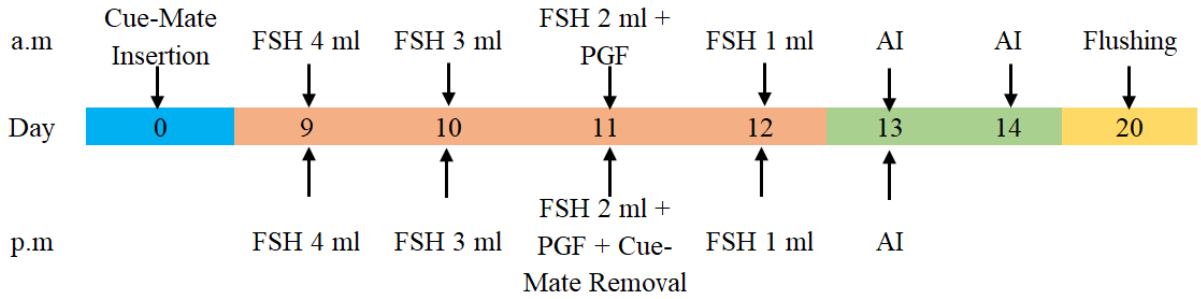

Figure 1. Superovulation protocol based on estrous synchronization

Table 2. Superovulation protocol based on estrous synchronization

Day	Time	Procedures	Description
0	a.m	Cue-Mate insertion	2 pods = 1.56 g progesterone
9	a.m	Treated with 4 ml FSH	FSH Dosage 400 mg/20 ml solvent
	p.m	Treated with 4 ml FSH	Intramuscular
10	a.m	Treated with 3 ml FSH	Intramuscular
	p.m	Treated with 3 ml FSH	Intramuscular
11	a.m	Treated with 2 ml FSH dan PGF2α	Prostaglandin Dosage 2 ml PGF2α
	p.m	Treated with 2 ml FSH, PGF2α, Cue Mate® removal	Intramuscular
12	a.m	Treated with 1 ml FSH	Intramuscular
	p.m	Treated with 1 ml FSH	Intramuscular
13	p.m	First Artificial Insemination (AI)	Interval 8-12 hours
14	a.m	Second AI	Interval 8-12 hours
	p.m	Third AI	Interval 8-12 hours
20	a.m	Identification of corpus luteum	by transrectal palpation
		Flushing and administration of PGF2α	Dosage 2 ml PGF2α
			Ringer Lactate 1,500 ml contains Bovine Serum Albumin and antibiotics

x ½ *Bos indicus* (Belgian Blue x Ongole Grade) were used as treatments. Superovulation refers to the method described by Jodiansyah et al. (2013). Donors were synchronized with 1.56 mg intravaginal progesterone (Cue-Mate®, Bioniche Animal Health Pty. Ltd, Australia) for 11 days, followed by intramuscular injection of 5 mg etiproston in 2 ml prostaglandin (Prostavet C®, Virbac Animal Health, France). In addition, breeds were superovulated with 400 mg NIH-FSH-P1 (Folltrophin V, Bioniche Animal Health Pty.

Ltd, Australia), which was administered for 4 days (a.m:p.m= 4ml:4ml; 3ml:3ml; 2ml:2ml; 1ml:1ml). Donors were inseminated three times (two days after progesterone implant removal in the morning and evening and three days after in the morning) with 0.25 ml of frozen-thawed semen. Belgian Blue semen was used for both Belgian Blue donors (purebred Belgian Blue embryo) and cross-bred (produces ¾ Belgian Blue x ¼ Ongole embryo). In contrast, Ongole grade semen was used for Ongole donors. The non-surgical embryos were flushed on day 7 after the first insemination (on day 20 of the estrous cycle). The estrous synchronization-based superovulation protocol is summarized in Figure 1 and Table 2.

Oocytes/embryo collection and evaluation

Feces were removed from the rectum and perineum before flushing the embryos. The donors were caged, and 4 ml of 2% Lidocaine Hydrochloride was administered for epidural anesthesia between sacrum-coccygeal I or coccygeal I-II to reduce peristalsis and discomfort. They were then assessed by rectal palpation before embryo retrieval, and the corpus luteum was estimated. Embryo flushing was performed 7 days after the first insemination. Donors received uterine lavage with a two-way Folley catheter size 18FR (FHK Fujihira, Jepang). The ringer's lactate was used as the lavage medium containing 1% bovine serum (Sigma-Aldrich, USA) and 100 IU/ml of 0.1% antibiotics penicillin-streptomycin (Sigma-Aldrich, USA). The volume of the embryo collection medium is reduced to approximately 10 ml using a 75 µm diameter filter (Agtech Inc., USA). Then, the results were transferred to a 100x100 mm petri dish (Falcon, USA). The oocytes/embryos collected in the medium are examined and evaluated using a stereomicroscope (Olympus SZ61, Japan) at a magnification of 50-100 times.

Oocytes/embryos were evaluated according to the IETS guidelines (Stringfellow and Givens 2010). Figure 2 shows the pre-implantation development of embryos in the blastocyst (stage 6) from different donor breeds. They were graded according to their quality, with grades 1, 2, 3, and 4 representing Excellent or Good, Fair or Regular, Poor, and Dead or Degenerate, respectively. Transferable (viable) embryos were categorized as grades 1, 2, and 3, while unfertilized and degenerated were defined as untransferable or grade 4 (non-viable). Each stage and grade is briefly described in the IETS manual (Bo and Mapletoft, 2013). Sales et al. (2008) proposed an index for embryo quality, namely the Embryo Quality Index (EQI). This index calculates the oocytes/embryos in each grade with their classification (number of Excellent*1)+(number of Good *2)+(number of Fair*3)+(number of Poor*4)+(number of Degenerate*5)+(number of unfertilized ova*5)/total amount of oocytes/embryos obtained.

Reproductive variables

The reproductive variables measured were the number of oocytes/embryos retrieved, recovery rate, fertilization rate, unfertilized oocytes, degenerated, development stage, grade, and proportion of transferable

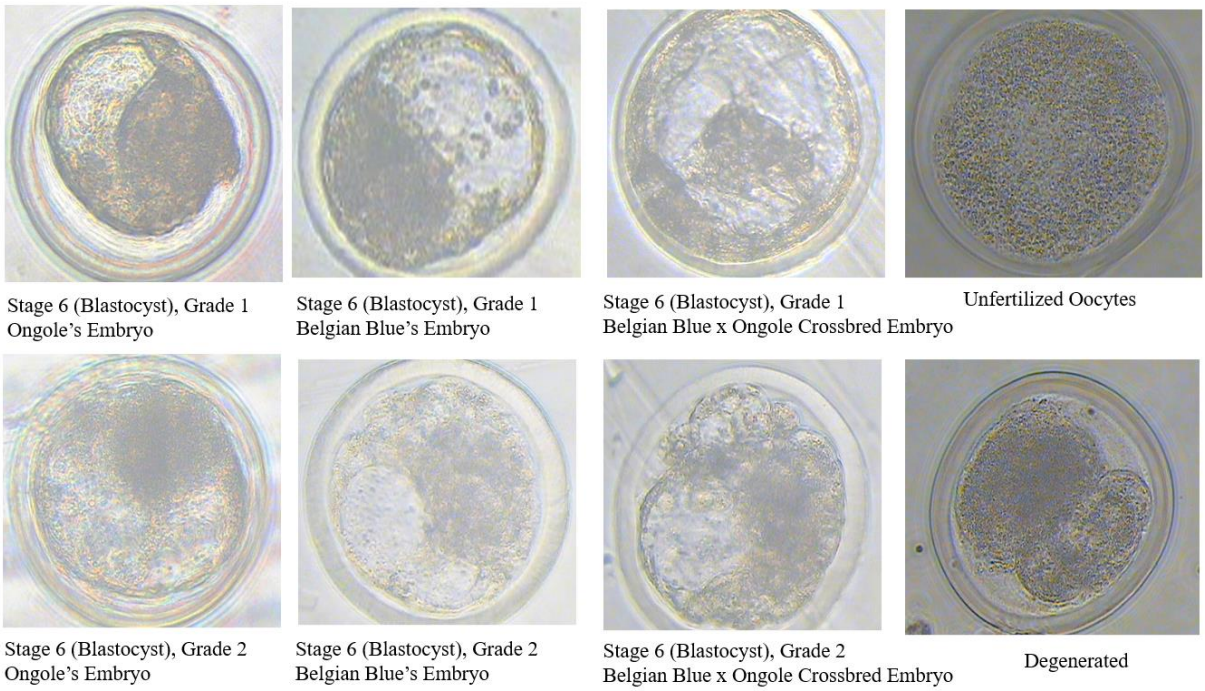

Figure 2. Development of embryos pre-implantation stage

embryos. The following equation was used to calculate the recovery rate, proportion of transferable embryos (viable embryos), and fertilization rate (Sumantri et al. 2011).

$$Recovery\ rate = \frac{Number\ of\ oocyte\ and\ embryo}{Number\ of\ corpus\ luteum} x100\%$$

$$Viable\ embryo = \frac{Grade\ 1,2,3}{Total\ oocyte\ and\ embryo\ recovered} x100\%$$

$$Fertilization\ rate = \frac{Grade\ 1,2,3, Degenerate}{Grade\ 1,2,3\ Unfertilize} x\ 100\%$$

Statistical analysis

The dependent variables were tested for normality using Kolmogorov-Smirnov and Saphiro-Wilk. Analysis was conducted using IBM SPSS Statistics for Windows, Version 26 (IBM Corp., Armonk, NY, USA). The comparison of breed effect on variables observed was analyzed using the Kruskal Wallis test. In addition, the pairwise mean test and significance were set at 95% ($P<0.05$). The following formula shows the model for the Kruskal-Wallis test.

$$H = \frac{12}{N(N+1)} \sum_{i-1}^{k} \frac{R_i^2}{ni} - 3(N+1)$$

where N is the sum of sample sizes in all samples, k is the number of samples, R_i is the sum of ranks in the i^{th} sample, and n_i is the size of the i^{th} sample.

RESULTS AND DISCUSSION

Superovulation response

The recovery rate was significantly different between Ongole grade and Belgian Blue ($P<0.05$) (Figure 3). Ongole grade showed a high recovery rate (89.63%). Oocytes/embryos recovered from Ongole in this study was 11.83±1.91, higher than the result of Imron (2016), which is 9.7±4.9. The main result of the present study showed that Ongole grade cows have the highest recovery rate. Belgian Blue showed a lower recovery rate (75.35%); the average oocytes/ embryos recovered was 4.86±1.33. According to Leroy et al. (2005) and Darlian et al. (2021), superovulated Belgian Blue showed a recovery rate of 87.3% and 75.80%, respectively (Table 3). The difference could be due to climate (subtropic and tropic), physiology (heifers and cows), or the superovulation method. Leroy et al. (2005) used ovulated oocytes as the superovulation response in Belgian Blue raised in subtropic. The superovulation response (calculated as the number of recovered, transferable, degenerated embryos, and unfertilized oocytes), transferable embryos, degenerated embryos, and unfertilized oocytes from Belgian Blue donors raised in the subtropic (numbers and percentage) were 9.2±0.8, 4.2±0.4 (45%), 1.1±0.2 (12%), and 3.9±0.7 (42%), respectively (Leroy et al. 2005).

The cross-bred in this study resulted in a 79.86% recovery rate, which showed no significant difference from its purebred (Table 3). The average oocytes/embryos recovered from cross-bred was 6.53±1.54. Research by Darlian et al. (2021) showed that embryos obtained in vivo from double-muscled crosses between Belgian Blue x Simmental and Belgian Blue x Holstein raised in the tropics did not have different recovery rates with purebred. Garcia et al. (2020) reported that cross-bred produced more oocytes/embryos (11.3±2.0). The optimal heterosis is expected in cross-breeding (Weaber 2015) and is optimized in ½:½ composition (Kirkpatrick 2017). According to Data Retrieval Committee IETS, in 2020 in vivo embryo of bovids was 10 oocytes with 6.2 transferable per flush worldwide (Viana 2021), which indicates low superovulation efficiency (Lonergan et al. 2016). Other factors affecting the superovulation response include the number of follicles in the ovary (Redhead et al. 2018) and the age of the donor (Landry et al. 2016). Donors over 9 years of age produce fewer embryos because fewer follicles to ovulate could respond to exogenous gonadotropins (Landry et al. 2016). The optimal age of the donor is less than 5 years (Jaton et al. 2016); in this study, it was 2-6 years age.

The fertilization rate was different between Ongole grade and Belgian Blue. The Ongole grade showed a high fertilization rate (77.35%) than Belgian Blue (68.22%) and the cross-bred (62.16%). Many unfertilized oocytes (37.84%) affected the fertilization rate in cross-bred cattle. The cross-bred cattle in this study were inseminated with purebred Belgian Blue semen to produce ¾ *Bos taurus* x ¼ *Bos indicus* embryos for upgrading purposes in the nucleus breeding scheme. According to O'Callaghan et al. (2021), sires did not affect fertilization rates, while Marsico et al. (2019) found that significant differences were observed depending on the sire. Research by Sukirman et al. (2019) confirms that the sire breed affects the quality of the semen produced, especially the motility for the fertilization process. In addition, donors in this study were inseminated three times to achieve optimum fertilization. To improve the fertilization rate, in this study, artificial insemination was synchronized in time (Figure 1).

Pre-implantation embryo development stage

As shown in Figure 4, the stage of pre-implantation embryo development (morula to hatched) was not different in all donor breeds. Based on Table 3, the percentage of embryos developed to blastocyst (stage 6) ranged from 13.54% to 26.17% (19.27% cumulative). The percentage of viable embryos that developed (morula to hatched) varied from 49.57% to 54.20%

(52.30% cumulative). In addition, total embryo cleavage (viable and degenerated) ranged from 62.16% to 75.90% (72.19% cumulative). The blastocyst rate of Belgian Blue in this study is higher (19.27%) than reported by Leroy et al. (2005), which is 17.3%.

Pre-implantation embryo development is a complex process involving molecular and structural changes. As development proceeds, control is switched from maternal to embryo-derived transcripts and proteins (Graf et al. 2014). Sudano et al. (2014) reported a higher blastocyst rate in Nelore (a type of Ongole cattle) than in Simmental. Residiwati et al. (2020) note the effect of the Belgian Blue Cross on pre-implantation development. The cross with Limousin resulted in better in vitro embryo development and quality than pure Belgian Blue or Limousin.

Belgian Blue showed fewer degenerated embryos than Ongole grade but higher than the crosses. Higher degenerate embryos in *Bos indicus* could be due to greater lipid droplet composition, which increases sensitivity to oxidative stress and disturbs mitochondrial function (Sudano et al. 2012). *Bos indicus* embryos generated *in vivo* and *in vitro* have a dark appearance, indicating a high lipid composition than taurine (Sudano et al. 2012). In contrast, the pale color of Belgian Blue indicates a lower lipid content (Leroy et al. 2005); this could likely be due to lipid metabolism in double-muscled cattle, but this issue requires further investigation. In this study, the crossing could increase the recovery rate and decreases the percentage of the degenerated embryo, but at the same time, it decreases the fertilization rate. However, those differences were not significant. According to Silva-Santos et al. (2014), a high prevalence of follicular atresia may lead to poor embryo development. In addition, hormones used in superovulation could cause oocyte atresia due to their function inducing follicular development and maturation (Chu et al., 2018).

Table 3. The total number of oocytes/embryos retrieved, recovery rate, embryo development, fertilization rate, embryos quality, and transferable embryos between donors breed (total numbers (percentage, %))

Description	Ongole grade	Cross-bred	Belgian Blue	Total
Total oocytes/embryo recovered (Recovery rate)	415 (89.63)[a]	111 (79.86)[ab]	107 (75.35)[b]	633
Fertilization				
Fertilized	321 (77.35)[a]	69 (62.16)[ab]	73 (68.22)[b]	463 (73.1)
Unfertilized oocytes	94 (22.65)	42 (37.84)	34 (31.78)	170 (26.85)
Development stage				
One cell	94 (22.65)	42 (37.84)	34 (31.78)	170 (26.85)
Morula	13 (3.13)	3 (2.70)	2 (1.87)	18 (2.84)
Early blastocyst	36 (8.67)	3 (2.70)	14 (13.08)	53 (8.37)
Blastocyst	79 (19.04)	15 (13.54)	28 (26.17)	122 (19.27)
Expand blastocyst	73 (17.59)	30 (27.03)	14 (13.08)	117 (18.48)
Hatched	11 (2.65)	4 (3.60)	0 (0)	15 (2.36)
Degenerated	103 (24.82)[a]	14 (12.61)[ab]	15 (14.02)[b]	132 (20.85)
Depeloved and viable (morula to hatched)	212 (51.08)	55 (49.57)	58 (54.20)	325 (52.30)
Cleavage (viable and degenerated)	315 (75.90)	69 (62.16)	73 (68.22)	457 (72.19)
Quality				
Grade 1 (Excellent)	168 (40.48)	39 (35.14)	34 (31.78)	241 (38.07)
Grade 2 (Fair)	49 (11.81)	14 (12.61)	19 (17.76)	82 (12.95)
Grade 3 (Poor)	1 (0.24)	2 (1.80)	5 (4.67)	8 (1.26)
Grade 4 (Discard)	197 (47.47)	56 (50.45)	49 (45.80)	302 (47.71)
Transferable Embryos	218 (52.53)	55 (49.55)	58 (54.20)	331 (52.29)

Different superscripts in the same row showed a significant effect ($P<0.05$)

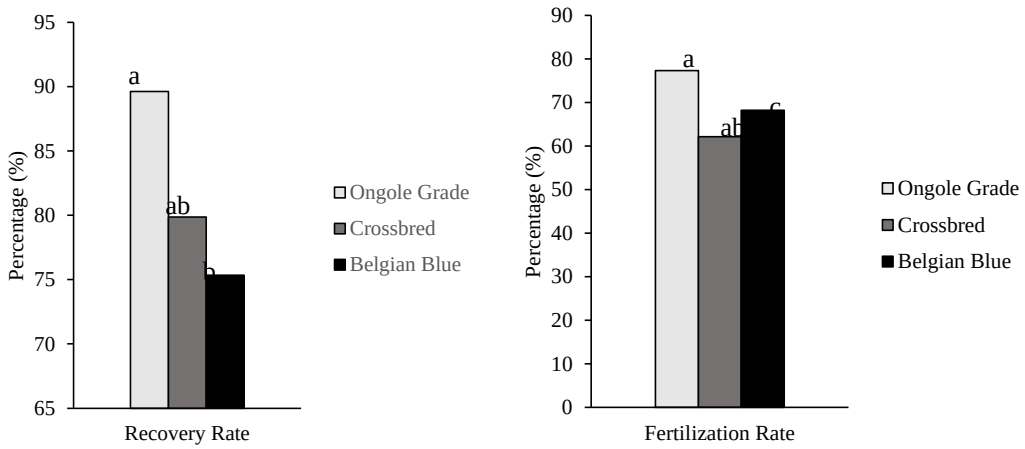

Figure 3. The proportion of recovery rate and fertilization rate from Ongole grade (light bars), cross-bred (gray bars), and Belgian Blue (black bars)

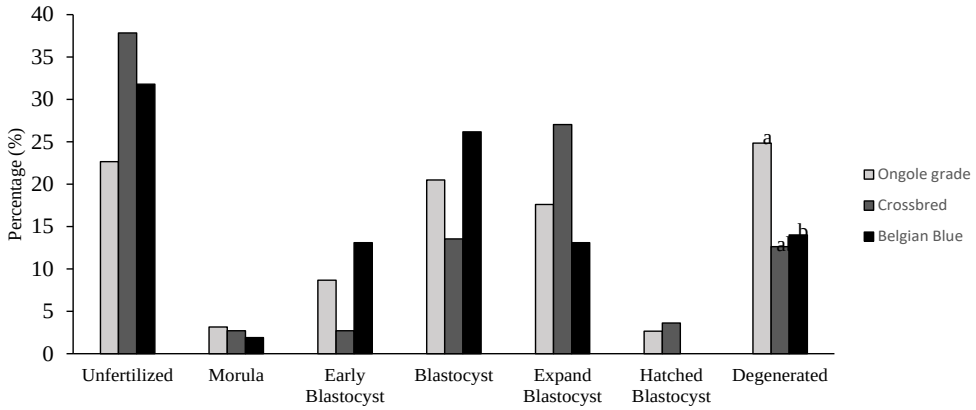

Figure 4. The proportion of development stage from Ongole grade (light bars), cross-bred (gray bars), and Belgian Blue (black bars)

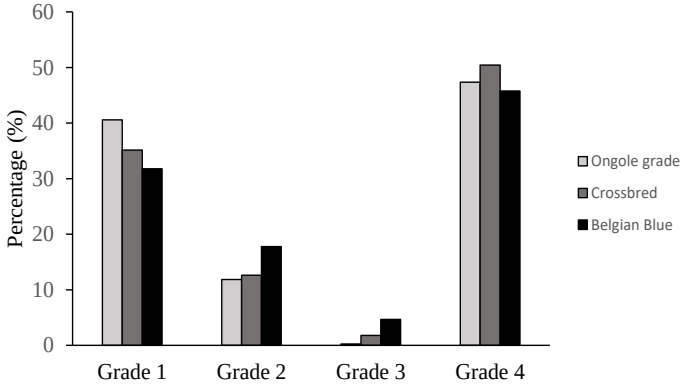

Figure 5. The proportion of embryo quality from Ongole grade (light bars), cross-bred (gray bars), and Belgian Blue (black bars

Other factors related to embryonic development becoming degenerated may correlate with developmental arrest in the early cleavage phase. Embryo molecular competence, associated with development arrest, occurs during the fourth or fourth and fifth cell cycle transition. Maternal RNA or proteins stored in the oocytes are degraded, and the embryonic genome is gradually activated (Graf et al. 2014). Bovine embryonic genome activation occurs between 8 and 16 cells (Graf et al. 2014). Problems during the pre-implantation period, failure of blastocyst hatching, inadequate cell cleavage, and death of the inner cell mass of embryos are all caused by disruption of the endoplasmic reticulum (Luo et al. 2006); this is because the endoplasmic reticulum is the primary mechanism associated with the stress response pathway and disruptions cell function, including cell cleavage (Latham 2015).

Embryo quality

In most mammalian species, especially cattle and humans, the morphological method is the oldest and most widely used in practice to evaluate embryo quality for grading systems. Embryo competence and quality are essential characteristics related to pregnancy rate in embryo transfer and can be explained by molecular, cellular, and morphofunctional factors. The IETS-recommended embryo morphological assessment system specifies several essential variables, including the number and shape of blastomeres, damaged cells, compaction, color, and development stage. This assessment is a standard international reference for embryo trade under the unified and simplified classification system. The Embryo Quality Index (EQI) proposed by Sales et al. (2008) was applied in this study. It yielded identical values ranging from 2.54 to 2.68, with values closer to 1 (one) indicating higher quality embryos (Sales et al. 2008).

In this study, the percentage of embryo quality of each grade was not different for all donor breeds. Based on Table 3, the percentage of the transferable embryos (grades 1, 2, and 3) varied between donors from 49.55% to 54.20% (52.29% cumulative). Belgian Blue produced 54.20% transferable embryos, with 45.80% being grade 4 discarded (unfertilized and degenerated). Naranjo-Chacon et al. (2019) reported that degenerated embryos and unfertilized oocytes from cross-bred with different ages (4-6 years and 8-12 years) showed similarities. A higher proportion of grade 4 was due to a high proportion of unfertilized oocytes (37.84%). Leroy et al. (2005) reported that superovulated Belgian Blue scored 55% Excellent grades, while in this study, only 31.78% were classified as Grade 1 (Excellent).

The high rate of degenerative embryos in superovulated donors may be due to abnormalities in oocyte maturation (Peralta-Torres et al. 2017). Makarevich et al. (2016) reported that the body condition score of donors affects pre-implantation embryo quality. A donor with a BCS of more than 4 produces a poor embryo, as indicated by severely degenerated cells and a fragmented embryo.

The cytoplasmic lipid content of the embryo is another aspect that affects embryo quality. Due to the low lipid content, the Belgian Blue embryo has a transparent appearance (Leroy et al. 2005). According to Ordonez-Leon et al. (2014), the cytoplasmic lipid of *Bos taurus* embryos was higher than *Bos indicus*. Nelore embryos had higher phospholipids (mainly phosphatidylcholine), which gave them a darker appearance than Simmental embryos (Visintin et al. 2002). Since *Bos taurus* was naturally selected under cold temperatures, this could affect oocyte and lipid composition in the embryo (Sudano et al. 2012). Marsico et al. (2021) demonstrated that triacylglycerol predominates in *Bos taurus* embryos. Triacylglycerol forms lipid droplets in the cytoplasm and serves as an energy source and cell signaling pathway for mammalian embryonic development.

CONCLUSION

This study concluded that: (1). Oocytes/embryos were successfully obtained from all donor cattle breeds with the production of oocytes/embryos from Ongole grade higher than Belgian Blue. (2). The cross-bred donor had identical oocyte and embryo production and quality compared to the purebred.

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