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In Vivo Embryo Production at Cipelang Livestock Embryo Centre

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INTRODUCTION

Livestock Embryo Center/Balai Embrio Ternak (BET) Cipelang is one of the government institution under the supervision of Directorate General of Livestock and Animal Health Services, Ministry of Agriculture of the Republic of Indonesia. BET Cipelang has the main task of carrying out the production, development, and distribution of livestock embryo for all regions of Indonesia. BET Cipelang produced embryos using two methods, there are *in vivo* and *in vitro*. The produced embryos will be transferred to the recipient cow using embryo transfer technique.

Embryo transfer biotechnology can be used to enhance genetic improvement and to increase marketing opportunities with purebred cattle. Because of their relatively low reproductive rate and long generation interval, embryo transfer is especially useful with cattle. The success of embryo transfer depends on factors associated with the embryo, the recipient or an interaction among the factors of the embryo and recipient [1].

Embryo quality is one of the main factors for successful embryo production. *In vivo* embryo production has better quality than *in vitro* such as cell number, morphology, growth and development ability and after freezing. The frequency of occurrence of chromosomal abnormalities in *in vivo*-derived embryos are lower than *in vitro* and the consequence is that only 30-40% of oocytes resulting from *in vitro* maturation develop into blastocysts after *in vitro* fertilization and the *in vitro* yield rate of embryos are lower than from *in vivo* [2]. Recently, there are no studies about the performance of *in vivo* embryo production at BET Cipelang. The aim of this study is to explore the quality of *in vivo* embryos produced by BET Cipelang.

MATERIALS AND METHODS

This study was considered using secondary data obtained from BET Cipelang. The data were an annual final report BET Cipelang from 2014 until 2017. The data obtained were the number of embryo grade 1,2,3 (transferable), degenerated, unfertilized and the total number of embryos collected. Data was presented in the number of embryos, the means of embryo/superovulation and the percentage of embryos.

RESULTS AND DISCUSSION

Assessment of Embryo quality: In BET Cipelang, Assessment of embryo quality based on the stage of embryonic development and the quality grade of the embryos. The standard of assessment of embryo quality has referred to standard assessment for embryo quality by the *International Embryo Transfer Society* (IETS). Embryo quality was determined by visual assessment of embryos morphological characteristics. This visual assessment was a subjective evaluation. The best predictor of the viability of an embryo was its stage of development relative to the given day after fertilization [3]. Some of the characteristics that can affect the quality of an embryo were regularity of shape the embryo, compactness of blastomeres (the dividing cells within the boundaries of the embryo), variation in cell size, color and texture of the cytoplasm (the fluid within the cell wall), overall diameter of the embryo, presence of extruded cells, regularity of the zona pellucida (the protective layer of protein and polysaccharides around the single-celled embryo), presence of vesicles (small bubble-like structures in the cytoplasm) [4]. The stage of embryonic development was stage 1 until 9 (unfertilized, 2 to 12 cells, early morula, morula, early blastocyst, blastocyst, expanded blastocyst, hatched blastocyst, and expanding hatched blastocyst, respectively). The grade of embryo quality was an excellent or good (grade 1), fair (grade 2), poor (grade 3) and dead or degenerating (grade 4) (figure 1). The criteria of transferable embryos were an embryo with stage 4 (morula) until stage 7 (expanded blastocyst) and grade 1

until grade 2. Grade 3 of embryos should only be transferred fresh or considered to culture for the next development. However, according to [5], stage 6 and 7 have higher pregnancy rates than stage 4,7 and 8 in Holstein heifers.

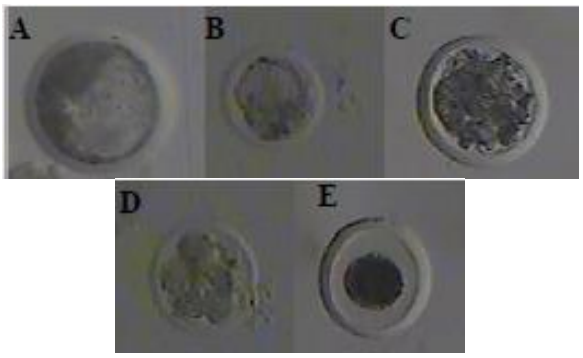

Figure 1. Microscopic picture of the embryo with grade 1 – 4 and unfertilized ova. A: grade 1, B: grade 2, C: grade 3, D: grade 4, E: unfertilized ova [2].

In vivo embryo production: *In vivo* embryo production at BET Cipelang has several steps, including the selection of the donor cow (performance, health, and reproductive status), superovulation for the donor cow, AI in the donor cow, and harvesting embryos (flushing). Harvested embryos were classified based on the result of embryo quality assessment. *In vivo* embryo production at BET Cipelang from 2014 until 2017 is available in Table 1.

Table 1. *In vivo* embryo production at BET Cipelang, Bogor from 2014 until 2017

Year	Grade of embryo (Number of the embryo; the mean of embryo/Superovulation (%Grade))			Total number of Embryo Production	The mean oocyte-embryo collected
	Transferable (123)	Degenerated	Unfertilized ova		
2014	716; 2.9 (36.1%)	547; 2.6 (27.2%)	719; 2.9 (36.3%)	1982	7.96
2015	1237; 2.9 (45.3%)	683; 1.6 (25.0%)	811; 1.9 (29.7%)	2731	6.5
2016	960; 3.8 (50.2%)	318; 1.3 (16.6%)	636; 2.6 (33.2%)	1914	7.69
2017	877; 3.9 (49.5%)	407; 1.8 (23.0%)	488; 2.1 (27.5%)	1772	7.8

Percentage of transferable embryos increased from 2014 to 2016 and decreased in 2017. In 2017, grade of the transferable embryo is different from other years, it is grade 1 and 2 in 2017 and grade 1,2 and 3 in the other years. According to [5], embryos of excellent and good (grade 1), or fair (grade 2) quality grade yield the highest pregnancy rates. A mean of transferable embryos is lower than in America, In America, the means of the transferable embryo were 7.0 embryo/superovulation for beef (>24,000 donors) and 6.3 embryo/superovulation for the diary (>15,000 donors) (data from the annual census of the AETA in 2011). That is influenced by many donor factors, such as breed, age, parity, and reproductive history. In addition, FSH preparation and superovulation protocol, climate, nutrition, and management factors can influence superovulation outcome. Of no less importance, however, are semen quality and the timing of AI and skill the inseminator [1].

Untransferable embryos ware caused by unfertilized ova and degenerated embryo on the female reproductive tract. Based on Table 1, the number of untransferable embryos is quite large. Unfertilized ova may be due to disturbances in sperm, ovarium transport, and sub-optimal oocyte quality [6]. Moreover, the greater dry matter intake of lactating cows increases the metabolism of estradiol leading to follicular persistence and reduce quality oocyte [7]. The higher number of percentage of degenerated embryos may be due to the higher incidence of poor fertilization results [6]. Several attempts were made to reduce the percentage unfertilized ova, such as conducting AI only at the time of signs estrus [is] clearly observed, refreshing the ovulation and increasing the AI frequency to 4-5 times.

CONCLUSION

The mean of *in vivo* embryo production and the transferable embryo in BET Cipelang is increasing every year. Several attempts have been made to reduce the untransferable embryo.

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