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Effects of Age and Season on *in vivo* Embryo Production of Friesian Holstein Cows

[1]Nurul Isnaini, [1]Syafira Aldina Febrianty, [1]Faizal Andri, [2]Edwar Edwar and [2]Fahrudin Darlian

[1]*Faculty of Animal Science, Universitas Brawijaya, Malang, Indonesia*
[2]*Indonesian Livestock Embryo Center Cipelang, Directorate General of Livestock and Animal Health, Bogor, Indonesia*

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Corresponding Author:
Nurul Isnaini
Faculty of Animal Science,
Universitas Brawijaya, Malang,
Indonesia
Email: nurulisna@ub.ac.id

Abstract: Embryo transfer is one of the leading technologies that is now being applied in an effort to increase the population and productivity of dairy cattle in Indonesia. Several factors may affect embryo production including age and season. In this study, the effects of age and season on *in vivo* embryo production were evaluated on Friesian Holstein (FH) cows. A total of 74 embryo production records from 10 FH cows aged 25-120 months were included in this study. The age of FH cows was divided into four categories, namely 25-48, 49-72, 73-96 and 97-120 months. The factor of season was divided into two categories, namely rainy (October-April) and dry (May-September). The variables observed were the number of corpus luteum, total embryo/ova, transferable embryo, degenerative cells, and nonfertilized oocytes. The results showed that the corpus luteum, total embryo/ova, and degenerative cells were not affected by age. However, the age significantly affected transferable embryos and nonfertilized oocytes, with the optimum range of age 49-72 months for transferable embryos. On the other hand, the season had no significant effect on all embryo production parameters. It could be concluded that age is a significant determinant for *in vivo* embryo production of FH cows. Meanwhile, the different seasons do not alter embryo production, which indicates that embryo collection can be performed throughout the year.

Keywords: Corpus Luteum, Dairy Cows, Embryo Transfer, Superovulation, Tropical Area

Introduction

The increase in the Indonesian population along with the increase in knowledge and public awareness toward balanced nutrition has resulted in increased demand for animal-based protein, including milk. The national milk demand in 2022 was about 0.99 million tons. This number was predicted to be increased in 2026 to become 1.07 million tons. However, domestic milk production can only fulfill about 22%, while another 78% should be imported from other countries (CADIS, 2022).

The low domestic milk production is probably related to the stagnant dairy cow population along with the low productivity. According to the DGLAH (2021), the dairy cow population in 2019 was 565 thousand heads, with a small increase in 2021 become 579 thousand heads. In terms of productivity, the milk production of dairy cows in Indonesia was only around 13.03-15.08 kg/day (Hartanto *et al*., 2020), which was tremendously low as compared to those in the subtropical area with milk production around 30.93-41.16 kg/day (Marumo *et al*., 2022).

The effort to increase dairy cow population and productivity is highly crucial to improving domestic milk production. Embryo transfer is one of the leading technologies that is now being applied to cope with this issue. The use of embryo transfer is beneficial to increasing the conception rate, especially in repeat-breeder dairy cows (Yaginuma *et al*., 2019; Nowicki, 2021). Additionally, embryo transfer also allows the production of progeny with high genetic merit from both cows and bulls sides (Mapletoft, 2013; Crowe *et al*., 2021). From these perspectives, it could be stated that embryo transfer is a valuable technique to improve dairy cow population and productivity. However, it should be pointed out that several factors such as age and season may influence embryo production. Therefore, the aim of this study was to evaluate the effects of age and season on *in vivo* embryo production of Friesian Holstein (FH) cows kept in tropical conditions in Indonesia.

Materials and Methods

Location

This study was carried out at the Indonesian livestock embryo center Cipelang (Bogor, Indonesia). The site is situated in the tropics at 6.706810' South Latitude and 106.771535' East Longitude, with an altitude of about 1240 m above mean sea level. The mean temperature and relative humidity in the site ranged from 18-22°C and 70-80%, respectively.

Donor Cows and Management

A total of 10 FH cows aged from 25-120 months were recruited from the Indonesian livestock embryo center in Cipelang, Indonesia for this study. The body weight and body condition score of the donor cows ranged from 550-650 kg and from 3.0-3.5, respectively. The donor cows used in this study had a normal reproductive cycle (18-21 days), had superior genetics, had no history of reproductive and other diseases, had given birth at least once, minimum at 90 days postpartum, and had proven fertility. These donor cows were fed 30-40 kg/head/day of elephant grass (*Pennisetum purpureum*) and supplemented with 5 kg/head/day concentrate with a minimum crude protein content of 16%. The provision of drinking water was done ad libitum. Donor cows were kept in a free stall housing system.

In vivo Embryo Production

In vivo, embryo production in the Indonesian livestock embryo center Cipelang was conducted in accordance with ISO 9001:2015 quality management system and Standard National Indonesian (SNI) 7880.1:2013. Estrous synchronization was carried out on day 1 by inserting a progesterone-releasing intravaginal device (Cue-Mate®, Bioniche Animal health (A/Asia) Pty. Ltd., Armidale, Australia). Superovulation was performed by injecting follicle-stimulating hormone (Folltropin V®, Bioniche Animal health (A/Asia) Pty. Ltd., Armidale, Australia) intramuscularly. The injection of follicle-stimulating hormone was done in the morning and evening starting from days 10-13 with reduced doses from 4, 3, 2, and 1 mL, respectively. After that, prostaglandin (Lutalyse®, Zoetis animal health, NJ, USA) was injected intramuscularly on day 12. The injection of prostaglandin was done twice in the morning and evening followed by the release of Cue-Mate®. On day 14, the donor cow will be in heat, and artificial insemination was carried out thrice with an interval of 8-12 h. On day 22, the embryos were harvested by using a non-surgical flushing method (Lubis *et al*., 2021). The number of corpus luteum and embryo/ova were recorded. The embryo quality was divided into transferable embryos, degenerated cells, and unfertilized oocytes in accordance with the international embryo transfer society (Robertson and Nelson, 2010). A total of 74 embryo production records from 10 FH cows were involved in the statistical analysis. The age of FH cows was divided into four categories, namely 25-48, 49-72, 73-96 and 97-120 months. The season was divided into two categories, namely rainy (October-April) and dry (May-September).

Statistical Analysis

Data on *in vivo* embryo production were statistically analyzed by analysis of variance using a general linear model. Data were presented as estimated marginal means ± standard error of means. p-values lesser than 0.05 were considered significant levels. Duncan's post-hoc test was used to separate means. Statistical analysis was performed using IBM SPSS statistics version 22.

Results and Discussion

The average number of corpus luteum, total embryo/ova, transferable embryo, degenerative cells, and nonfertilized oocytes of FH cows in this study were 8.31±0.77, 6.91±0.79, 3.45±0.53, 1.70±0.34, 1.76±0.36 per collection, respectively. In comparison with other breeds, the *in vivo* embryo production of FH cows in this study was relatively similar as compared to Belgian blue crossbred and Ongole grade cattle. It was reported that the number of corpus luteum, total embryo/ova, transferable embryo, degenerative cells, and nonfertilized oocytes of Belgian blue crossbred cattle were 8.42, 6.08, 3.83, 1.33, and 0.92 per collection, respectively (Darlian *et al*., 2021). In Ongole grade cattle, the number of corpus luteum, total embryo/ova, transferable embryo, degenerative cells, and nonfertilized oocytes were 8.90, 8.90, 7.86, 1.00 and 2.30 per collection, respectively (Lubis *et al*., 2021). However, the total embryo/ova and transferable embryo in this study was slightly lower as compared to Nellore, Simmental, and Brahman cattle. It was reported that Nellore cattle produce 10.8 embryos/ova with 5.5 transferable embryos (Silva *et al*., 2009). In Simmental cattle, the total embryo/ova and transferable embryo were 12.80 and 9.11, respectively (Lubis *et al*., 2021). In a study by Martínez *et al*. (2021), Brahman cattle produced 15.99 embryos/ova with 9.83 transferable embryos. The different regions and climatic conditions, along with the differences in the genetics of cows may be attributed to the difference in the *in vivo* embryo production.

Table 1: Effect of age on *in vivo* embryo production of Friesian Holstein cows

Age (months)	Corpus luteum	Total embryo/ova	Transferable embryo	Degenerative cells	Nonfertilized oocytes
25-48	6.68±1.14	5.30±1.18	2.03±0.75[a]	1.95±0.53	1.33±0.50[a]
49-72	10.44±1.54	8.94±1.60	6.22±1.01[b]	1.42±0.72	1.31±0.68[a]
73-96	9.70±2.20	8.33±2.28	3.28±1.44[ab]	1.23±1.02	3.83±0.97[b]
97-120	9.53±2.39	8.23±2.48	2.37±1.57[ab]	2.17±1.11	3.70±1.06[ab]

Data were presented as estimated marginal means ± standard error of means
[a,b]different superscripts within a column indicate significant differences ($p<0.05$)

Table 2: Effect of season on *in vivo* embryo production of Friesian Holstein cows

Season	Corpus luteum	Total embryo/ova	Transferable embryo	Degenerative cells	Nonfertilized oocytes
Rainy	9.94±1.23	8.35±1.28	4.18±0.81	1.44±0.57	2.73±0.54
Dry	8.24±1.43	7.06±1.48	2.77±0.93	1.94±0.66	2.36±0.63

Data were presented as estimated marginal means ± standard error of means

The effect of age on *in vivo* embryo production of FH cows is presented in Table 1. The results showed that the number of corpus luteum, total embryo/ova, and degenerative cells were not affected by age ($p>0.05$). However, the age significantly affected the number of transferable embryos and nonfertilized oocytes ($p<0.05$). A remarkable increase in the number of transferable embryos was evidenced when FH cows were at 49-72 months ($p<0.05$). After this point, a numerical reduction in transferable embryos was observed but without significant differences ($p>0.05$). In terms of the number of nonfertilized oocytes, this parameter was lower when FH cows at 25-48 and 49-72 months as compared to those at 73-96 and 97-120 months. According to this finding, it could be stated that the most optimum age for *in vivo* embryo production of FH cows was 49-72 months.

In agreement with this finding, Naranjo-Chacón *et al*. (2019) also reported that the age of the donor had a significant effect on *the in vivo* embryo production of *Bos taurus* × *Bos indicus* cows ($p<0.05$). A previous study by Villaseñor González *et al*. (2017) also showed that age was one of the essential factors in the *in vivo* embryo production of Criollo Coreño cattle. The lower number of transferable embryos in FH cows aged 25-48 months is probably related to the lower corpus luteum and total embryo/ova, which subsequently provide a lower number of transferable embryos. As described by Villaseñor González *et al*. (2017), the young cows had lower responses to superovulation because of endocrine failures due to the high concentration of circulating estradiol. Parallel to this current result, Jaton *et al*. (2016) also observed the numerical reduction of embryo production in the donor cows at 7 years old onward.

The effect of season on *in vivo* embryo production of FH cows is presented in Table 2. The results showed that the number of corpus luteum, total embryo/ova, transferable embryo, degenerative cells, and nonfertilized oocytes were all not affected by the season ($p>0.05$). This finding indicates that the different seasons did not alter *in vivo* embryo production so that the embryo collection of FH cows can be performed throughout the year.

In line with this finding, the amount of transferable embryos of FH cows also was not affected by the different seasons (Lee *et al*., 2012). Martínez *et al*. (2021) also found that the tropical seasons had no significant effect on the number of transferable embryos and degenerative cells of Brahman cows. No significant effect of season on *in vivo* embryo production parameters probably because of no extreme change in the ambient temperature as well as relative humidity throughout the year (Isnaini *et al*., 2021). In the previous study, it was indicated that the change in the ambient temperature and relative humidity between two seasons in Indonesia (rainy and dry) was only 0.3°C and 0.8%, respectively and this situation did not alter reproductive performance in Simmental cattle Isnaini *et al*. (2019).

Conclusion

It could be concluded that age has a significant contribution to the *in vivo* embryo production of FH cows, with the optimum age for transferable embryos ranging from 49-72 months. Meanwhile, the different seasons do not alter embryo production, which indicates that embryo collection can be performed throughout the year. More research on the impact of donor age and season on embryo transfer success is strongly urged.

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Author's Contributions

Nurul Isnaini: Conception and design, analysis, and interpretation of data reviewed the article critically for significant intellectual content and gave final approval of the version to be submitted and any revised version.

Syafira Aldina Febrianty and Faizal Andri: Conception and design, acquisition, and analysis of data drafted the article, and gave final approval of the version to be submitted and any revised version.

Edwar Edwar and Fahrudin Darlian: Conception and designed, acquired, and interpretation of data, reviewed the article critically for significant intellectual content, and gave final approval of the version to be submitted and any revised version.

Ethics

This article is original and contains unpublished material. The corresponding author confirms that all of the other authors have read and approved the manuscript and no ethical issues involved.

References

CADIS. (2022). Outlook Susu (Indonesian). Ministry of Agriculture of the Republic of Indonesia, Jakarta. *Center for Agriculture Data and Information Systems*. https://satudata.pertanian.go.id/assets/docs/publikasi/FINAL_OUTLOOK_SUSU_2022.pdf

Crowe, A. D., Lonergan, P., & Butler, S. T. (2021). Invited review: Use of assisted reproduction techniques to accelerate genetic gain and increase value of beef production in dairy herds. *Journal of Dairy Science*, *104*(12), 12189-12206. https://doi.org/10.3168/jds.2021-20281

Darlian, F., Wahjuningsih, S., Rosmayanti, A., Jodiansyah, S., Jalaludin, L. A., Setiawan, Y., & Susilawati, T. (2021). Respon superovulasi sapi persilangan Belgian Blue dengan metode yang berbeda. *Jurnal Agripet*, *21*(2), 178-186. https://doi.org/10.17969/agripet.v21i2.20407

DGLAH. (2021). Livestock and Animal Health Statistics 2021. Directorate General of Livestock and Animal Health. *Ministry of Agriculture of the Republic of Indonesia, Jakarta*. http://www.ditjenpkh.pertanian.go.id/buku-statistik-peternakan-dan-kesehatan-hewan-tahun-2021

Villaseñor González, F., Torre Sánchez, J. F. D. L., Martínez Velázquez, G., Álvarez Gallardo, H., Pérez Reynozo, S., Palacios Fránquez, J. A., ... & Montaño Bermúdez, M. (2017). Characterization of the ovarian response to superovulation in Creole Coreno cattle using reduced doses of FSH. *Revista Mexicana de Ciencias Pecuarias*, *8*(3), 225-232. https://doi.org/10.22319/rmcp.v8i3.4498

Hartanto, R., Pamungkas, A. A., Prayitno, E., & Harjanti, D. W. (2020). Milk Production of Holstein Friesian Dairy Cows in Various Lactation Periods (Case Study at Capita Farm, Semarang, Central Java). *Jurnal Ternak vol*, *11*(2), 44-49. https://doi.org/10.30736/jy.v11i2.73

Isnaini, N., Lestari, B. D. W. andri, F., Harsi, T. and Sukmawati, E. (2021). Association between climatic factors with fresh semen quality parameters in Madura cattle (an Indonesian native breed). *American Journal of Animal and Veterinary Sciences*, *16*: 185-191. https://doi.org/10.3844/ajavsp.2021.185.191

Isnaini, N., Wahjuningsih, S., & Adhitama, E. (2019). Seasonal effects on semen quality of Ongole crossbred and Simmental bulls used for artificial insemination. *Livest. Res. Rural Dev*, *31*(2), 16. http://www.lrrd.org/lrrd31/2/nurul31016.html

Jaton, C., Koeck, A., Sargolzaei, M., Malchiodi, F., Price, C. A., Schenkel, F. S., & Miglior, F. (2016). Genetic analysis of superovulatory response of Holstein cows in Canada. *Journal of Dairy Science*, *99*(5), 3612-3623. https://doi.org/10.3168/jds.2015-10349

Lee, W., Song, K., Lim, K., Lee, S., Lee, B., & Jang, G. (2012). Influence of factors during superovulation on embryo production in Korean Holstein cattle. *Journal of Veterinary Medical Science*, *74*(2), 167-174. https://doi.org/10.1292/jvms.11-0057

Lubis, A. F., Satyaningtijas, A. S., Lubis, O. P., Kurniati, W., & Boediono, A. (2021, November). Superovulation response of Peranakan Ongole (PO) and Simmental cows after FSH stimulation in multiple ovulation and embryo transfer program. In *IOP Conference Series: Earth and Environmental Science* (Vol. *902*, No. 1, p. 012044). IOP Publishing. https://doi.org/10.1088/1755-1315/902/1/012044

Mapletoft, R. J. (2013). History and perspectives on bovine embryo transfer. *Animal Reproduction (AR)*, *10*(3), 168-173. https://animal-reproduction.org/article/5b5a6048f7783717068b468e

Martínez, J. F., Galina, C. S., Ortiz, P., Maquivar, M. G., & Romero-Zúñiga, J. J. (2021). Effects of Season on Donor and Recipient Cows and Calf Performance from Birth to Weaning in Embryo Transfer Programs in the Tropics. *Animals*, *11*(12), 3596. https://doi.org/10.3390/ani11123596

Marumo, J. L., Lusseau, D., Speakman, J. R., Mackie, M., & Hambly, C. (2022). Influence of environmental factors and parity on milk yield dynamics in barn-housed dairy cattle. *Journal of Dairy Science*, *105*(2), 1225-1241. https://doi.org/10.3168/jds.2021-20698

Naranjo-Chacón, F., Montiel-Palacios, F., Canseco-Sedano, R., & Ahuja-Aguirre, C. (2019). Embryo production in middle-aged and mature Bos taurus× Bos indicus cows induced to multiple ovulation in a tropical environment. *Tropical Animal Health and Production*, *51*, 2641-2644. https://doi.org/10.1007/s11250-019-01975-2

Nowicki, A. (2021). Embryo transfer as an option to improve fertility in repeat breeder dairy cows. *Journal of Veterinary Research*, *65*(2), 231-237. https://doi.org/10.2478/jvetres-2021-0018

Robertson, I., & Nelson, R. E. (2010). Certification and identification of embryos. *Manual of The International Embryo Transfer Society. 4th Ed, Champaign: International Embryo Transfer Society*, 86-105.

Silva, J. C. C., Alvarez, R. H., Zanenga, C. A., & Pereira, G. T. (2018). Factors affecting embryo production in superovulated Nelore cattle1. *Animal Reproduction (AR)*, *6*(3), 440-445. https://www.animal-reproduction.org/article/5b5a6070f7783717068b4775

Yaginuma, H., Funeshima, N., Tanikawa, N., Miyamura, M., Tsuchiya, H., Noguchi, T., ... & Hamano, S. (2019). Improvement of fertility in repeat breeder dairy cattle by embryo transfer following artificial insemination: Possibility of interferon tau replenishment effect. *Journal of Reproduction and Development*, *65*(3), 223-229. https://doi.org/10.1262/jrd.2018-121