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Analysis of Embryo Transfer Pregnancy Rate from Friesian Holstein Cow Recipients in BET Cipelang

Analisis Tingkat Kebuntingan Transfer Embrio dari Penerima Sapi Friesian Holstein di BET Cipelang

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ABSTRAK

Indonesia memiliki potensi besar dalam sektor peternakan sapi potong dan sapi perah, namun menghadapi tantangan dalam peningkatan kualitas genetik ternak. Transfer embrio merupakan bioteknologi yang dapat meningkatkan kualitas genetik dan produktivitas ternak. Penelitian ini bertujuan untuk mengkaji dan menganalisis faktor-faktor yang mempengaruhi keberhasilan tingkat kebuntingan pada sapi perah FH sebagai penerima transfer embrio di BET Cipelang. Data sekunder dari BET Cipelang (2017-2023) digunakan, meliputi kualitas embrio, metode transfer, umur penerima, metode sinkronisasi estrus, dan ras donor embrio. Hasil menunjukkan bahwa kualitas embrio yang baik (24,55%) dan metode transfer segar (35,19%) secara signifikan meningkatkan keberhasilan kehamilan ($p < 0,05$). Metode sinkronisasi menggunakan PGF2α dan kombinasi Progesteron + PGF2α meningkatkan peluang kebuntingan masing-masing 3,60 dan 3,89 kali dibandingkan estrus alami. Meskipun tidak signifikan secara statistik, kelompok umur 3-5 tahun dan 6-9 tahun memiliki peluang lebih tinggi untuk kebuntingan dibandingkan dengan kelompok umur 10-12 tahun. Ras donor embrio tidak menunjukkan perbedaan signifikan terhadap tingkat kebuntingan. Penelitian ini menyarankan pentingnya perhatian pada kualitas embrio dan metode transfer untuk meningkatkan tingkat keberhasilan transfer embrio.

KEYWORDS:
Dairy cow
Embryo
Embryo transfer
Estrus
Pregnancy

ABSTRACT

Indonesia has significant potential in the beef and dairy cattle sectors but faces challenges in improving the genetic quality of livestock. Embryo transfer is a biotechnology that can enhance genetic quality and livestock productivity. This study aims to examine and analyze the factors affecting the success rate of pregnancies in FH dairy cattle as recipients of embryo transfer at BET Cipelang. Secondary data from BET Cipelang (2017-2023) were used, including embryo quality, transfer methods, recipient age, estrus synchronization methods, and embryo donor breeds. The results showed that good embryo quality (24.55%) and fresh transfer methods (35.19%) significantly increased pregnancy success ($p < 0.05$). Synchronization methods using PGF2α and a combination of Progesterone + PGF2α increased the chances of pregnancy by 3.60 *and 3.89 times, respectively, compared to natural estrus. Although not statistically significant, the age groups of 3-5 years and 6-9 years had higher chances of pregnancy compared to the 10-12 years age group. The donor breed of embryos did not show a significant difference in pregnancy rates. This study highlights the importance of focusing on embryo quality and transfer methods to improve embryo transfer success rates.*

1. Introduction

Indonesia has significant potential in the livestock sector, particularly in the development of beef and dairy cattle. However, the main challenge lies in the availability of superior breeds that can enhance productivity and the quality of livestock products (Balehegn et al., 2020; Terry et al., 2021). Improving genetic quality is a strategic approach to overcome this challenge, as superior genetics can increase productivity, production efficiency, and reduce costs for farmers (Crowe et al., 2021). One effort made is through the application of Embryo Transfer (ET), a biotechnology aimed at improving genetic quality, productivity, and disease control in livestock (Yousuf et al., 2024). ET allows the use of high-quality embryos from superior female and male cattle, shortens the generational interval, and facilitates the import and export of embryos, in addition to increasing fertility in cattle experiencing reproductive difficulties.

However, in Indonesia, the pregnancy rate of ET is still relatively low, as reported by BET Cipelang in 2022, with a pregnancy rate of about 26.9% (Imron, 2023). This low pregnancy rate poses a challenge because high pregnancy rates in ET can provide significant financial efficiency and reduce costs associated with maintaining non-pregnant animals (Ferreira et al., 2021). The success of ET is influenced by various factors, including the skill of the ET operator, the stage and quality of embryo development, inconsistent estrus cycles between donors and recipients, high environmental temperatures, inadequate luteal function in recipients, and the use of frozen and fresh embryos (Lestari et al., 2019).

Analyzing these factors is crucial to increasing ET efficiency. Improving operator skills and managing environmental conditions, as well as selecting high-quality embryos and recipients, can enhance pregnancy rates and bring significant economic benefits to farmers (Khan et al., 2022). Thus, the synergy between genetic improvement and the optimization of the ET process is key to developing a more productive and efficient cattle farming sector in Indonesia. Previous studies with similarities to this research, such as

Damaris et al. (2023) and Study Marzouk et al. (2020), discussed the impact of embryo quality and estrus synchronization methods on the success of embryo transfer, similar to this study that analyzes embryo quality and estrus synchronization methods in FH cattle at BET Cipelang. Additionally, Thomson et al. (2021) and Kurniati et al. (2020) evaluated the characteristics of the corpus luteum (CL) in embryo recipients and their relationship to pregnancy success rates, which aligns with the data analysis in this research, including information on estrus synchronization methods and embryo quality.

However, this research differs from Damaris et al. (2023), which focused more on comparing the embryo production capabilities among various donor cattle breeds, while this study emphasizes the factors affecting pregnancy rates in FH recipient cattle at BET Cipelang. Thomson et al. (2021) concentrated on the influence of the physical characteristics of the CL on conception rates, whereas this research considers factors of embryo quality and estrus synchronization methods. Marzouk et al. (2020) focused on maiden recipient cattle and blood progesterone levels, while this research includes recipient cattle of various ages and estrus synchronization methods. Study Kurniati et al. (2020) explored estrus detection methods and estrus expression in frozen-thawed embryo transfer, whereas this study examines estrus synchronization methods and embryo quality in fresh and direct embryo transfers.

The novelty of this study lies in the comprehensive analysis of various factors affecting pregnancy rates in FH dairy cattle at BET Cipelang in the context of embryo transfer, including embryo quality, transfer methods, recipient age, estrus synchronization methods, and embryo breed. Therefore, the purpose of this research is to examine and analyze the factors influencing pregnancy rates in FH dairy cattle as recipients in embryo transfer at BET Cipelang, providing new insights into the optimal combination of these factors to enhance embryo transfer success rates, which have not been thoroughly discussed in previous studies. This can help in designing more effective strategies to increase milk and meat production in Indonesia through the application of livestock biotechnology.

2. Materials and Methods

This research did not require IRB/IACUC approval because there were no human and animal participants, as it involved the analysis of secondary data obtained from the

Livestock Embryo Center.

2.1.Time and Location

The research was conducted from October 2023 to December 2023 at the Livestock Embryo Center (BET) Cipelang, Bogor Regency, West Java.

2.2. Materials

The data used in this study are secondary data obtained from BET Cipelang from 2017 to 2023. The data collected include the quality of cattle embryos used, types of cattle embryos, pregnancy rates, age of recipient cows at ET, the transfer methods used (fresh or direct), and estrus synchronization methods for recipient cows. The recipient cows used were FH females (n=206) from BET, healthy, aged around 3-12 years, with a Body Condition Score (BCS) of 2.75-3, and not pregnant. These animals were managed intensively and fed with forages and concentrates according to their physiological needs. Fresh embryos were produced by BET Cipelang, while frozen embryos were obtained through import or from fresh embryos that had undergone cryopreservation. The direct method refers to the ability to transfer thawed frozen embryos directly to the recipient without the need for complex cryoprotectant dilution procedures or the use of a microscope and non-surgical recipient operations. Pregnancy examinations were conducted 2 to 3 months after ET using Ultrasound (USG) (Draminski 4Vet Portable Ultrasound®) equipped with a linear rectal transducer with B-Mode Doppler and a frequency of 5-10 MHz. Equipment used for uterine health examination included a spool gun, gun cover, plastic wrap, and syringes.

2.3. Methods

This study was conducted in several stages.

2.3.1. Recipient selection

Female cows that passed the selection process and met the recipient requirements for embryo transfer (ET) were as follows: 1) recipients were virgin or non-pregnant cows, with healthy reproductive organs and normal reproductive/estrous cycle records; 2) aged around 3-7 years; 3) in good physical condition and healthy with a Body Condition Score (BCS) of 2.75-3.25 on a scale of 5; 4) healthy and not showing symptoms of infectious diseases; 5) having a normal and functional Corpus Luteum (CL); 6) no more than two pregnancy failures (Lamb et al., 2021; Parlindungan, 2022).

2.3.2. Recipient preparation

Selected recipient candidates were prepared for the embryo transfer process by synchronizing the estrous cycle between donors, from which the embryos would be harvested (flushing), and recipients, which would be injected with hormones intramuscularly or into muscle tissue. The hormonal protocol used for estrus synchronization at BET Cipelang was as follows: (1) 7 days intravaginal progesterone + PGF2α, (2) Ovsynch GnRH-PG, (3) single injection of PGF2α, and (4) combination of PMSG-hCG. The synchronization method used in this study was prostaglandin (PGF2α) with a single injection and intravaginal progesterone (P4) for 7 days + PGF2α with a protocol as shown in **Figure 1.**

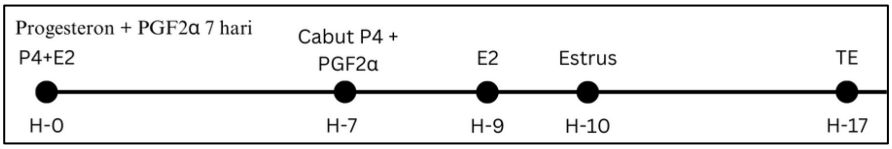

Figure 1. Protocol of 7 days of intravaginal progesterone + PGF2α

Natural synchronization was also performed if there were recipient cows experiencing estrus simultaneously with the donor. Estrous recipients were checked and their estrus condition observed, including the degree of estrus, as well as mucus consistency and clarity, which had to be normal and recorded. On the sixth or seventh day after estrus, the ovaries were re-examined to check the condition of the Corpus Luteum (CL). The CL must function well in either the left or right ovary to proceed with ET application.

2.3.3. Embryo transfer stage

Recipients prepared with estrous synchronization and having a functional CL could proceed to the ET application stage. First, recipient cows underwent epidural anesthesia using an anesthetic preparation. Embryos used for ET application were at the morula to hatching blastocyst stage and had the appropriate quality for transfer. Subsequently, the embryos in the straws were thawed after being taken from the container. ET application was done by placing the embryo in the anterior third of the horn with the CL (ipsilateral), as this location had the highest chance of pregnancy success (Baldassarre, 2021; Falchi et al., 2022).

2.3.4. Pregnancy Examination

Pregnancy examination was conducted 2 to 3 months after the ET procedure using ultrasound (USG). At this time, the embryos had developed sufficiently, making pregnancy detection more accurate and efficient.

2.4. Statistical Analysis

The proportion of pregnancy rates, defined as the number of positive pregnancy examinations (PE) from recipients relative to the total number of recipients who received embryo transfer, will be grouped based on observed variables. Results will be calculated based on previous studies by Sanches et al., (2016) and analyzed descriptively using the following formula:

$$\%\ \text{Pregnancy rate} = \frac{\sum PE\ Pregnant\ Recipient}{\sum Total\ of\ Recipient} \text{ X } 100\%$$

To determine the relationship between observed variables, namely embryo quality, embryo transfer method, recipient age, synchronization method, and embryo type, partial binary logistic regression adapted from Alkan et al. (2020) and Mora et al. (2020) will be analyzed using IBM® SPSS Statistics version 27 with a significance level of 5% and 1%.

3. Results and Discussion

3.1. Influence of Embryo Quality on Pregnancy Rate

The influence of embryo quality on pregnancy rate is calculated by determining the success percentage. The analysis to see the regression coefficient, significance value (P), and OR can be found in **Table 1.**

Table 1. Influence of embryo quality on pregnancy rate

Variable	Number (head)	Pregnant (head)	Not Pregnant (head)	% Pregnancy	b	S.E	P	OR
Embryo Quality								
Good (1)	167	41	126	24.55	1.15	0.55	0.036*	3.16
Fair (2)	39	8	31	20.51	Reference			
Total	206							

Information: [TN]not significant (p>0.05) * significant (p<0.05)

Embryo quality significantly influences pregnancy success rate (p < 0.05). Good quality embryos (1) have a higher success rate of 24.55% with an OR of 3.16 compared to fair quality (2), which is about 20.51%. This result is similar to previous studies, such as those by Alkan et al. (2020), which stated that quality 1 has a higher success rate (44.66%) compared to quality 2 (33.07%). Quality 1 embryos have at least 85% intact cell material and a live embryo mass compared to quality 2, which has at least 50% of cells and live embryo mass. Quality 1 embryos are better able to survive freezing/thawing procedures compared to quality 2 because the freezing process can reduce embryo quality. Quality 1 is recommended for international trade because it is more resistant to the freezing process (Pytlík et al., 2020).

3.2. Influence of Transfer Method on Pregnancy Rate

The transfer methods analyzed are direct and fresh. BET Cipelang uses a non-surgical direct transfer method that refers to the ability to transfer thawed embryos directly to recipients without complicated cryoprotectant dilution procedures or the use of microscopes and surgery on recipients (Gomes et al., 2020). The pregnancy rate is calculated based on the success percentage and analyzed to see the regression coefficient, significance value (P), and OR as shown in **Table 2.**

Table 2. Influence of transfer method on pregnancy rate

Variable	Amount (tail)	Pregnant (tail)	Not Pregnant (tail)	% Pregnancy	b	S.E	P	OR
Method								
Fresh	54	19	35	35.19	1.74	0.47	>.001*	5.68
Direct	145	34	111	23.45	Reference			
Total	206							

Information: [TN]not significant (p>0.05) * significant (p<0.05)

The embryo transfer method significantly influences pregnancy success rate ($p < 0.05$). OR indicates that using the fresh method (35.19%) has a 5.68 times higher chance of resulting in pregnancy compared to the direct transfer method (23.45%). This result aligns with Hansen, (2020), who stated that the cryopreservation process can reduce fresh embryo quality. A decrease in pregnancy percentage also occurs when using frozen embryos (20.1%) compared to fresh (31.2%) in research sites where cows are exposed to heat stress (Stewart et al., 2011). Frozen embryos have lower success due to osmotic stress, ice crystal formation, cold shock, damage to cell membranes from water-to-ice transition, or toxic cryoprotectants (Barbosa et al., 2023; Dochi, 2019). Fresh embryos are not exposed to these conditions, thus maintaining better cell integrity and function.

3.3. Influence of Age on Pregnancy Rate

The age of recipient cows is divided into three groups: 3-5 years, 6-9 years, and 10-12 years. Analysis is conducted to see the regression coefficient, significance value (P), and OR as shown in **Table 3.**

Table 3. Influence of age on pregnancy rate

Variable	Amount (tail)	Pregnant (tail)	Not Pregnant (tail)	% Pregnancy	b	S.E	P	OR
Age (years)								
3-5	66	18	48	27.27	0.91	0.69	0.18	2.5
6-9	116	27	89	23.28	0.74	0.67	0.27	2.1
10-12	24	4	20	16.67	Reference			
Total	206							

Information: ^{TN}not significant ($p>0.05$) * significant ($p<0.05$)

Age does not show a significant difference in pregnancy rates, but based on OR, the age groups 3-5 and 6-9 years have 2.5 and 2.1 times higher chances, respectively, of resulting in positive pregnancies compared to the 10-12 years group. Previous research showed an increase in pregnancy rates in cows aged 3-7 years and a decrease in success in cows older than 7 years (Shorten et al., 2015). Embryo transfer is recommended in adult cows, with experienced cows having higher pregnancy rates. In general, a good recipient selection should have healthy udders and be under 8 years old (Lamb et al., 2021).

3.4.Influence of Synchronization Method on Pregnancy Rate

The methods used for this analysis are natural estrous, single injection of PGF2α, and intravaginal Progesterone (P4) + PGF2α for 7 days. The results can be seen in **Table 4.**

Table 4. Influence of synchronization method on pregnancy rate

Variable	Number (tail)	Pregnant (tail)	Not Pregnant (tail)	% Pregnancy	b	S.E	Say.	OR
Sync. Method								
Natural estrous	159	32	127	20.13	Reference			
PGF2α 1x	30	11	19	36.66	1.28	0.51	0.01	3.60
P4 + PGF2α	17	6	11	35.29	1.36	0.66	0.04	3.89
Total	206							

Information: TN not significant ($p>0.05$) * significant ($p<0.05$)

The synchronization method of estrous in recipients shows significant differences in pregnancy rates ($p < 0.05$). Based on OR, PGF2α (36.66%) has a 3.60 times and Progesterone + PGF2α has a 3.89 times higher chance compared to natural estrous in resulting in pregnancy. Single injection of PGF2α has the lowest cost among all methods, but accurate estrous detection post-injection is required (Mukkun et al., 2021). PGF2α is effective for females with regular estrous cycles and CL, but not for those with irregular cycles. Previous research shows that using Progesterone + PGF2α has higher effectiveness and can be used in cows with abnormal cycles compared to single PGF2α injection, though the cost is higher (Hamd et al., 2023; Lamb et al., 2021; Saini et al., 2023).

3.5. Influence of Donor Breed on Pregnancy Rate

The embryos used in this study come from FH, Simmental, and Limousin donors. The analysis of pregnancy rates based on donor breed can be seen in **Table 5.**

Table 5. Effect of donor embryos breed on pregnancy rate

Variable	Amount	Pregnant	Not Pregnant	% Pregnancy	b	Say.	OR
Donor embryo							
FH	49	13	36	26.53	Reference		
Simmental	97	22	75	22.68	-0.73	0.107TN	0.48
Limousin	60	14	46	23.33	-0.94	0.062TN	0.39
Total	206						

The donor breed of embryos used does not have a significant difference in pregnancy rates ($p > 0.05$). Previous research by Lacerda et al. (2020) states that the breed of donor cows does not affect the success of embryo transfer to recipients of different breeds. This supports the concept that successful pregnancies are more related to inherent factors of the embryos, such as their quality, rather than the breed itself. According to Lestari et al. (2016), dairy cows can accept embryos from other breeds with a 55% success rate and no rejection from the immune system. Donor embryos can be recognized as foreign objects, causing rejection and miscarriage. Maternal recognition and pregnancy are influenced by the embryo through the secretion of interferon-tau (IFN-t). IFN-t is an antiluteolytic substance that prevents CL lysis by inhibiting PGF2α release from the uterus, thus reducing oxytocin receptors in the endometrium (Aréchiga et al., 2019). This leads to early embryo death as the embryo cannot produce IFN-t, causing rejection in the recipient.

4. Conclusion

This study shows that factors influencing the pregnancy rate of FH dairy cows as recipients in embryo transfer at BET Cipelang include embryo quality, transfer method, recipient age, estrus synchronization method, and donor breed. Good embryo quality (success rate of 24.55%) and the fresh transfer method (success rate of 35.19%) significantly affected the increase in pregnancy success ($p < 0.05$). The synchronization methods using PGF2α and a combination of Progesterone + PGF2α also increased pregnancy chances by 3.60 and 3.89 times, respectively, compared to natural estrus. Although not statistically significant, the age groups of 3-5 years and 6-9 years had higher chances of pregnancy compared to the 10-12 years group. Conversely, the donor breed did not show significant differences in pregnancy rates, supporting previous studies that success is more influenced by embryo quality than breed. This study indicates that to improve pregnancy rates in FH dairy cows, attention should be paid to embryo quality and the transfer method used. The study has several limitations, namely the use of secondary data, which may impact the quality and completeness of the data, thus affecting the results. Additionally, the study only used FH cows as recipients, so the results may not be generalizable to other cattle breeds. In the future, it is recommended to conduct longitudinal studies and involve various recipient cattle breeds to obtain more comprehensive results. Further research should also consider genetic and environmental factors in more detail to understand their effects on embryo transfer success.

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