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Genetic and non-genetic factors influencing sperm motilities of beef bulls during cryopreservation process

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ABSTRACT

This study aimed to evaluate genetic and non-genetic factors affecting motility after the cryopreservation process. Sperm motility records of five breeds of Bulls were obtained from Singosari National Artificial Insemination Center of Indonesia. A data set comprised 36754 records of ejaculation collected from 143 bulls. The records comprised progressive motility of fresh semen (PMFS), pre-freeze motility of liquid semen (PMLS), and post-thawing motility (PTM). The MIXED procedure with Tukey–Kramer multiple comparisons and Pearson correlation of Statistical Analysis System (SAS) was used for data analysis. This study shows that breed, season, age, and ejaculation influence the PMFS. In five breeds, the youngest bulls produced fresh semen with the lowest progressive motility. The highest PMFS occurs at high precipitation in Bali, Limousin, and Simmental. PMFS for Brahman and Ongole Grade was not significantly affected by precipitation. The first ejaculate is higher than the second ejaculate except for the Limousin and Simmental. PMLS and PTM were significantly different between the breeds. PMLS of Bali, and Limousine bulls were significant between different seasons, whereas that of Brahman, Ongole Grade, and Simental were not. The differences in the age of bulls were not shown in Bali bulls. PTM for all the breeds was significant between different seasons, except for Ongole Grade bull. All breeds showed the highest PTM in the season with medium precipitation, except for Ongole Grade bull. The highest post-thawing motility in five breeds was observed in the youngest bull. The heritability value of motility traits is included in the medium category (0.23 – 0.28). The phenotypic correlation between the motility of fresh semen, liquid semen, and frozen semen was low (0.14-0.15) but still correlated ($p<0.05$).

Introduction

Motility is one of the main parameters used to evaluate semen quality, along with concentration, morphology, and viability. High motility indicates healthy, active sperm capable of fertilizing an egg. Sperm motility is directly related to the chance of successful fertilization. In artificial insemination (AI), only semen with adequate post-thaw motility (at least 40%) is considered acceptable for use. One of the critical factors to ensure the success of AI is the quality of the semen produced at the AI center. The quality of semen that is considered to be a predictor of the success of AI is motility (Gebreyesus *et al*., 2021; Pardede *et al*., 2020). Motility testing in frozen semen production procedures is mandatory, when the semen is still fresh and after freezing according to the standard of Badan Standardisasi Nasional (2017).

Studying genetic and non-genetic factors influencing bull semen quality is crucial because there is significant variation in semen quality, influenced not only by the environment but also by heredity. This knowledge is needed to improve the effectiveness of AI, reproductive efficiency, and the success of livestock breeding programs. Semen motility can be influenced by external factors, such as the environment (Konenda *et al*., 2020; Corte Pause *et al*., 2022; Ramajayan *et al*., 2023), and internal factors, such as the breed of bulls (Berry *et al*., 2019; Landaeta-Hernández *et al*., 2020). Improving semen motility and durability after the cryopreservation process has a role in the production of quality frozen semen, which will directly increase the success of AI services. In addition, genetic variability across breeds on the impact of cryopreservation on semen motility provides knowledge of genetic factors and the interactions with the environment that can affect semen motility (Berry *et al*., 2019).

The ability of sperm to withstand the cryopreservation process is a key factor in selecting superior males at AI centers. Previous studies have shown that semen quality, particularly motility, declines after dilution and freezing (Amal *et al*., 2019; Konenda *et al*., 2020; Mostek *et al*., 2017). Therefore, motility is an essential aspect of semen quality that must be carefully assessed during frozen semen production, especially in AI centers. This study focused on evaluating both genetic and non-genetic factors influencing motility after the cryopreservation process in AI centers.

Materials and methods

Data collection

Sperm motility records of five breeds of Bulls were obtained from Singosari National Artificial Insemination Center of Indonesia. A data set comprised 36754 records of ejaculation collected from 143 bulls. The data was collected between January 2018 and December 2021, the records comprised of progressive motility of fresh semen (PMFS), pre-freeze motility of liquid semen (PMLS), and post-thawing motility (PTM). Semen was collected twice a week on Monday and Thursday. The fresh motility was evaluated immediately after semen was ejaculated. Pre-freeze motility was evaluated from the sample of semen straw before the cooling process. The post-thawing motility was evaluated one day after freezing by thawing samples of semen straw. Sperm motility was evaluated using Computer Assisted Sperm Analysis (CASA). The seasonal data was carried out from the Meteorology, Climatology, and Geophysical Agency of East Java, Indonesia.

Categories and Statistical Analysis

Five breeds used in the study were Simental (n=13764), Limossin (n=16979), Brahman (n=1442), Ongole Grade (n=1009), and Bali (n=3560) ejaculations. Seasons were classified into three categories based on monthly rainfall: low precipitation (0–50 mm, n = 14,259), medium precipitation (51–150 mm, n = 13,662), and high precipitation (151–>300 mm, n = 8,833). Bulls were grouped into six age categories: A1 (<3 years; n = 7,848), A2 (≥3–<4 years; n = 10,612), A3 (≥4–<5 years; n = 9,208), A4 (≥5–<6 years; n = 4,621), A5 (≥6–<7 years; n = 1,344), and A6 (≥7 years; n = 3,121). Ejaculations were further divided based on collection order: first ejaculation (n = 25,137) and second ejaculation (n = 10,151). A detailed summary of the data is presented in Table 1.

Table 1. Descriptive statistics of the data used in the study.

Variable	Number	Mean	SD	Minimum	Maximum
PMFS (%)	35211	77.12	9.43	55.5	98.9
PMLS (%)	26894	55.59	3.5	40	70
PTM (%)	24188	42.88	3.79	35	65.16
Age	36754	4	1.72	1.39	11.81
Temperature	768	25.85	2.73	21.37	31.57
Humidity	768	68.82	8.82	55.83	86.94

PMFS: Progressive Motility of Fresh Semen; PMLS: Progressive Motility of Liquid Semen; PTM: Post-Thawing Motility; SD: Standard deviation

The MIXED procedure with Tukey–Kramer multiple comparisons of Statistical Analysis System (SAS, 2021) was used to analyze the factors that affected sperm motilities. Bulls were treated as a random effect. The linear model was as follows:

$$Y_{ijklmno}=G_i+A_j+S_k+E_l+(GA)_{ijl}+(GS)_{ikm}+b_n+e_{ijklmno}$$

Where yijklmno is the observation of sperm motilities, G_i the ith fixed effect of breed, A_j the jth fixed effect of age, S_k the kth fixed effect of season, E_l the lth fixed effect of number of ejaculation, $(GA)_{ijl}$ and $(GS)_{ikm}$ are lth, and mth between ith of breed with jth effect of age and between ith of breed with kth effect of season, respectively, bn is the random effect of bull, and $e_{ijklmno}$ the random residual of $Y_{ijklmno}$. Pearson correlation was used for analysis the phenotypic correlation between semen motilities.

The mixed model analysis identified factors that influence male semen quality traits, which were then used to calculate additive genetic variance and residual variance for heritability estimation. These variance values were estimated using the Gibbs sampling method with the THRGIBBS1F90 software, part of the BLUP90 program family (Misztal *et al.*, 2015).

Gibbs sampling produces 5,000 sample rounds with a burn-in of 1000 samples and an interval of 10 samples. 400 post-Gibbs samples (POSTGIBBSF90) were used for the final analysis, calculating estimated heritability values for each trait. The linear model for genetic parameter analysis is as follows (Setiaji *et al.*, 2021):

$y=Xb+Gu+e,$

Where y vector of observations, b vector of fixed effect, X incidence matrices for fixed effect, u vektor of random additive genetic effect, G incidence matrices for random effect, and e vector of random residuals.

Results

The descriptive statistics showed that sperm motility decreased from fresh semen, pre-freeze, and post-thawing. Table 1, PMFS were associated with breeds, season, age of bulls, ejaculation, and their interaction ($p<0.05$). The difference between breeds was observed in all seasons, classes of age, and ejaculation. PMFS of Bali, Limousine, and Simental bulls were significant between different seasons, whereas that of Brahman, and Ongole Grade were not. In five breeds, the youngest bulls produced fresh semen with the lowest progressive motility. The differences in the age of bulls were shown in all breeds in Table 2. Table 3 presents the interaction between breeds and season, breed and age of bull, and breed and ejaculation for PMLS. PMLS were associated with breeds, season, age of bulls, ejaculation, and their interaction ($p<0.05$). The difference between breeds was observed in all seasons. PMLS of Bali, and Limousine bulls were significant between different seasons, whereas that of Brahman, Ongole Grade, and Simental were not. The differences in the age of bulls were not shown in Bali bulls. PTM was associated with breeds, season, age of bulls, ejaculation, and their interaction ($p<0.05$). The difference between breeds was observed in all seasons and classes of age. PTM for all of the breeds was significant between different seasons, except for Ongole Grade bull. All breeds showed the highest PTM in the season with medium precipitation. The highest post-thawing motility in five breeds was observed in the youngest bull. The differences in age of bulls were shown in all breeds in Table 4. The values in Table 5 present correlation results of each particular trait of semen motility. All the values of correlation coefficients received positive correlation values. In each breed, PMFS and PMLS correlation is the weakest, with correlation coefficient values ranging from 0.13 to 0.17. In similar manner, PMFS and PTM is also the same and the range of correlation coefficients is 0.05 to 0.15. PMLS and PTM are positively correlate d at and the range at 0.11 to 0.30. In the case of the Simmental, Limousin, and Bali and Ongole Grade breeds, the

Table 2. Progressive Motility of Fresh Semen from different breeds, season and age of bulls.

Factors	Mean	Breed				
		Bali	Brahman	Limousine	Ongole Grade	Simmental
Precipitation						
Low	77.74±0.54	69.10±1.11^{ax}	75.66±1.46^{y}	80.43±0.61^{az}	76.86±2.00^{yz}	79.07±0.65^{az}
Medium	78.63±0.54	72.13±1.11^{bx}	76.78±1.47^{y}	80.50±0.61^{abz}	76.61±2.01^{xy}	80.20±0.65^{bz}
High	79.06±0.54	72.90±1.11^{cx}	75.89±1.47^{y}	80.82±0.61^{by}	76.85±2.01^{xy}	80.77±0.65^{cy}
Age						
A1	70.96±0.54	60.22±2.47^{ax}	71.77±1.60^{ayz}	72.38±0.60^{az}	66.74±2.02^{ay}	73.78±0.62^{az}
A2	76.32±0.54	66.73±1.23^{bx}	71.61±1.39^{ay}	78.23±0.59^{bz}	75.19±1.99^{byz}	78.95±0.62^{bz}
A3	77.67±0.54	71.28±1.15^{cx}	75.18±1.43^{by}	79.72±0.59^{cz}	76.13±1.98^{byz}	79.69±0.62^{cz}
A4	78.37±0.55	75.67±1.21^{dx}	79.63±1.49^{cy}	80.04±0.61^{cy}	78.09±2.12^{cxy}	79.93±0.65^{cy}
A5	81.51±0.59	74.03±1.11^{dx}	80.48±1.64^{cy}	84.02±0.73^{dz}	81.24±2.27^{dyz}	83.53±0.87^{dyz}
A6	86.02±0.63	80.35±1.06^{ex}	77.99±2.58^{bcx}	89.12±0.85^{ez}	83.25±2.37^{dxy}	84.18±1.05^{dy}
Ejaculation						
	1 77.24±0.53	73.27±1.09^{bx}	77.40±1.38^{by}	79.20±0.60^{ay}	77.64±1.93^{by}	78.26±0.64^{ay}
	2 79.71±0.54	69.48±1.13^{ax}	74.82±1.57^{ay}	81.97±0.61^{bz}	75.91±2.10^{ay}	81.75±0.66^{bz}

[a-e]Values within a column without a common superscript are different (P < 0.05).
[x-w]Values within a row without a common superscript are different (P < 0.05).

Table 3. Progressive Motility of Liquid Semen from different breeds, season and age of bulls.

Factors	Mean	Breed				
		Bali	Brahman	Limousine	Ongole Grade	Simmental
Precipitation						
Low	55.53±0.18	55.84±0.37^{az}	54.88±0.50^{xy}	55.66±0.21^{byz}	55.50±0.67^{xyz}	54.81±0.22^{x}
Medium	55.37±0.18	55.84±0.37^{ay}	55.14±0.50^{xy}	55.36±0.21^{ay}	55.61±0.68^{y}	54.68±0.22^{x}
High	55.52±0.18	56.31±0.37^{bz}	55.33±0.50^{xy}	55.44±0.21^{ay}	55.71±0.68^{yz}	54.79±0.23^{x}
Age						
A1	55.44±0.18	55.54±0.93	54.73±0.56^{ab}	55.26±0.20^{a}	54.90±0.69^{a}	55.30±0.21^{c}
A2	55.26±0.18	55.91±0.43^{y}	54.46±0.47^{ax}	55.21±0.20^{axy}	55.06±0.67^{axy}	54.98±0.21^{bxy}
A3	55.47±0.18	56.28±0.39^{y}	55.18±0.48^{bxy}	55.46±0.20^{axy}	55.20±0.67^{axy}	55.09±0.21^{bx}
A4	55.54±0.19	55.72±0.42	55.51±0.51^{bc}	55.56±0.21^{b}	55.31±0.73^{a}	55.11±0.22^{bc}
A5	55.76±0.21	56.14±0.37^{x}	55.95±0.58^{cx}	55.74±0.27^{by}	57.46±0.79^{bx}	54.88±0.33^{bx}
A6	55.35±0.23	56.38±0.35^{y}	54.86±0.96^{abxy}	55.67±0.35^{by}	55.72±0.84^{ay}	53.21±0.42^{ax}
Ejaculation						
1	55.54±0.18	56.00±0.36^{y}	55.08±0.46^{xy}	55.57±0.20^{by}	55.99±0.64^{y}	54.79±0.22^{x}
2	55.40±0.18	56.00±0.38^{z}	55.15±0.55^{xy}	55.40±0.21^{ayz}	55.22±0.73^{xyz}	54.73±0.23^{x}

$^{a\text{-}c}$Values within a column without a common superscript are different (P < 0.05).
$^{x\text{-}w}$Values within a row without a common superscript are different (P < 0.05).

Table 4. Post-Thawing Motility from different breeds, season and age of bulls.

Factors	Mean	Breed				
		Bali	Brahman	Limousine	Ongole Grade	Simmental
Precipitation						
Low	41.81±0.19	43.62±0.41^{az}	40.42±0.55^{ax}	42.01±0.23^{ay}	41.71±0.73^{xy}	41.35±0.25^{axy}
Medium	42.46±0.19	44.53±0.41^{bz}	41.24±0.56^{bx}	42.70±0.23^{by}	41.91±0.74^{xy}	41.86±0.25^{bx}
High	41.74±0.19	43.88±0.41^{az}	40.44±0.56^{ax}	41.90±0.23^{ay}	41.84±0.75^{xy}	41.17±0.25^{ax}
Age						
A1	42.59±0.20	44.28±1.02^{by}	41.69±0.63^{bxy}	42.64±0.22^{bxy}	41.54±0.75^{abx}	42.28±0.23^{cxy}
A2	42.84±0.19	44.17±0.46^{bw}	41.05±0.52^{abx}	43.10±0.22^{cy}	42.23±0.74^{bxy}	42.44±0.23^{cy}
A3	42.23±0.19	44.43±0.42^{bz}	40.80±0.53^{abx}	42.49±0.22^{by}	41.76±0.73^{bxy}	41.70±0.23^{bx}
A4	41.45±0.20	44.57±0.46^{bz}	40.52±0.57^{abx}	41.62±0.23^{ay}	40.85±0.79^{axy}	40.83±0.24^{ax}
A5	41.43±0.22	43.30±0.40^{ay}	40.64±0.64^{abx}	41.38±0.29^{ax}	42.59±0.86^{bxy}	40.84±0.37^{ax}
A6	41.50±0.25	43.31±0.38^{az}	39.49±1.06^{ax}	41.99±0.39^{aby}	41.94±0.91^{bxy}	40.66±0.51^{ax}
Ejaculation						
1	42.16±0.19	44.13±0.40^{z}	40.94±0.51^{x}	42.38±0.22^{by}	42.06±0.70^{xy}	41.55±0.24^{x}
2	41.85±0.20	43.89±0.42^{z}	40.45±0.61^{x}	42.03±0.24^{ay}	41.58±0.80^{xy}	41.37±0.26^{xy}

$^{a\text{-}c}$Values within a column without a common superscript are different (P < 0.05).
$^{x\text{-}w}$Values within a row without a common superscript are different (P < 0.05).

Table 5. Pearson Correlations between fresh, liquid and post-thawing motilities in each breeds.

Correlation	Breed				
	Simmental	Limousin	Bali	Ongole grade	Brahman
PMFS-PMLS	0.16164**	0.16602**	0.12936**	0.17187**	0.17156**
PMFS-PTM	0.14114**	0.13780**	0.15180**	0.14928**	0.05
PMLS-PTM	0.24653**	0.22386**	0.29906**	0.25840**	0.11358**

PMFS, Progressive Motility of Fresh Semen; PMLS, Progressive Motility of Liquid Semen; PTM, Post-Thawing Motility.

correlation is weak and rest of the other breeds, stronger. The heritability of motility traits falls within the medium range (0.23–0.28).

Discussion

The average PMFS of 77.12% that we found in this study was slightly higher than the PMFS reported in previous studies. For example, a PMFS of 67% has been reported for Brahman (Corbet *et al*., 2013), 65 % for Angus (Menegatti Zoca *et al*., 2020), 39.33% for Nelore and Angus (Hidalgo *et al*., 2021), 64.22 and 63.54 % for Limousin and Simmental (Hapsari *et al*., 2022), 66.8, 67.2 and 68.9% for Limousin, Simmental, and Bali bulls, respectively (Isnaini *et al*., 2019). The bulls of SNAIC were strictly selected from hundreds of superior bulls based on individual performance and pedigree analysis. A good quality diet and routine exercise have been carried out to maintain the optimal body weight of bulls. The outcome of the controlled program may have increased the quality of sperm, especially progressive motility.

The effect of precipitation on PMFS can be seen in Bali, Limousin, and Simmental breeds where the highest PMFS occurs at high precipitation. This result is in accordance with Brito *et al*. (2002) which state that

Limousin and Simental have high adaptability in the tropics. Meanwhile, PMFS for Brahman and Ongole Grade was not significantly affected by precipitation. This could be due to the body shape of Brahman and Ongole Grade having a wattle which can help them carry out the process of evaporation (releasing heat) more than other breeds, so the effect of precipitation is relatively small. Konenda *et al.* (2020) stated that there was a relationship between temperature and the continuity of spermatogenesis, where heat can reduce motility, especially motility when spermatozoa were in the epididymis (Vogler *et al.*, 1991). PMFS increases with age. This was consistent with several studies which state that age had a significant effect on motility (Bhakat *et al.*, 2011; Boujenane & Boussaq, 2013; Suyadi *et al.*, 2020).

The effect of ejaculate on PMFS in each breed is significantly different, the first ejaculate is higher than the second ejaculate, except for the Limousin and Simmental breeds which have higher motility in the second ejaculate. Motility is higher in the first ejaculation because the second ejaculation which is carried out on the same day does not have enough time to produce spermatozoa of the same quality as the first ejaculation. Longer ejaculation intervals will produce better semen (Boujenane & Boussaq, 2013), and lower semen quality can be caused by short ejaculation intervals (Murphy *et al.*, 2018).

The average PMLS of 55.59% from five breeds of bulls was in the range of 51.1 to 62.1 % reported in Limousin, Madura, Bali, and Ongole Grade (Ratnawati *et al.*, 2018; Konenda *et al.*, 2020). On the other hand, the average PTM of 42.88% was slightly lower than 46.25% for Simmental (Susilowati *et al.*, 2021) and 52-55% for Holstein Friesian bulls (Murphy *et al.*, 2018). Besides management for the bull, PMLS and PTM were affected by the cryopreservation process. Dilution of semen in suitable buffers is important to maintain sperm motility during preservation (Rasul *et al.*, 2000). The buffer conditions used in this research on each breed are the same because the research was in one area.

PMLS and PTM showed significant differences across breeds, suggesting that genetics play a role in determining these values. Correlation analysis revealed genetic variability influencing the relationship between semen motility before and after storage (cryopreservation). Berry *et al.*, (2019) reported a very strong genetic correlation between semen motility prior to and following cryopreservation. Both PMLS and PTM are strongly affected by the integrity of the spermatozoa membrane (Konenda *et al.*, 2020), which can be damaged during the dilution process (Khalil *et al.*, 2018) and freezing (Amal *et al.*, 2019).

The phenotypic correlation in this study (0.14–0.15) is slightly lower than what was reported for Nordic Holstein bulls (0.19) (Gebreyesus *et al.*, 2021). These findings suggest that the phenotypic correlation between PMFS and PTM is minimal and can essentially be disregarded. This indicates that while PMFS shows a positive association with PTM, the relationship is not strong (Gebreyesus *et al.*, 2021). The weak genetic correlation may be influenced by several factors beyond the phenotypic and genetic aspects already mentioned, particularly the cryopreservation process. This process involves standard operating procedures (SOPs), the equipment used, and the personnel carrying it out (Setiaji *et al.*, 2021). Earlier studies have demonstrated that post-cryopreservation motility is positively and strongly linked to the Non-Return Rate (NRR) in males (Gebreyesus *et al.*, 2021), whereas fresh semen motility does not show this relationship (Mapel *et al.*, 2022).

The heritability estimates in this study were calculated using genetic variance and residual values generated by the program (Wijaya *et al.*, 2023). These estimates are considered reliable, as indicated by the Posterior Standard Deviation (PSD) for both additive genetic variance and residual variance being smaller than the variance values themselves (Ambike *et al.*, 2022). The results obtained are generally consistent with previous studies (Burren *et al.*, 2019; Berry *et al.*, 2019; Mandal *et al.*, 2020) and are higher than those reported by Ambike *et al.* (2022). However, this estimate is somewhat lower compared to findings from earlier studies, which reported values between 0.32–0.44 (Corbet *et al.*, 2013), 0.37 (Berry *et al.*, 2019) and 0.32 (Gebreyesus *et al.*, 2021).

Motility traits are known to be associated with fertility, and higher heritability indicates more genetic variability (Ambike *et al.*, 2022). So genetic selection through this motility trait is expected to improve and increase the fertility of semen produced by each beef cattle bull in Indonesia. The heritability estimates for motility in this study align well with the average values reported by Berry *et al.* (2019). According to Ambike *et al.* (2022), low heritability estimates suggest that meaningful genetic progress can only be achieved if additional information from relatives is incorporated into the selection process.

The various breeds used in this study represent the genetic variability that exists among the superior bulls of the AI center. Although phenotypically, the relationship between PMFS and PTM is low, the quality of fresh semen still influences the quality of frozen semen. Genetically, it has been proven by several studies that the genetic correlation of pre- and post-cryopreservation semen motility is higher than the phenotypic correlation (Gebreyesus *et al.*, 2021) and very high (Berry *et al.*, 2019). The importance of choosing bulls with good genetic material and how maintaining the quality of fresh semen during the cryopreservation process is the best way for AI centers to produce quality frozen semen.

Conclusion

This study shows that breed, season, age, and ejaculation influence the motility of fresh semen. Semen motilities after freezing and thawing were significantly different between the breeds. The phenotypic correlation between the motility of fresh semen, liquid semen, and frozen semen was low but still correlated.

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Conflict of interest

The authors have no conflict of interest to declare.

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