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Repeatability and Phenotypic Correlation Among Semen Quality Traits in Holstein Bulls

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ABSTRACT

The purpose of this research was to analysis the value of repeatability and correlation among the traits affecting the production of frozen semen from Holstein's bull in Indonesia. Repeatability and correlation were calculated based on the data of frozen semen production of 15.699 records from 44 Holstein bulls at Singosari Artificial Insemination Center (SAIC) and 8.935 records from 39 Holstein bulls at Lembang Artificial Insemination Center (LAIC). Repeatability for volume, motility, fresh semen concentration and frozen semen production was evaluated by intraclass correlation method. The repeatability values of LAIC for volume, motility, fresh semen concentration and frozen semen production were 0.60; 0.54; 0.37 and 0.47. The repeatability values of SAIC for volume, motility, fresh semen concentration and frozen semen production were 0.54; 0.30; 0.43 and 0.29. The linear correlation value between volume, motility and fresh semen concentration with the amount of semen produced per collections were 0.41, 0.36, and 0.58. Concentration was the most factors influencing the number of frozen semen produced. The effectiveness of the selection of Holstein's frozen semen producing could be determined by the value of repeatability and the phenotypic correlation among semen quality traits such as volume, motility, concentration and frozen semen production.

Keywords: Phenotypic correlation, Repeatability, Semen production, Selection

Introduction

Successful rate of artificial insemination (AI) in the farmer levels are affected by many factors, one of these factors is the quality of frozen semen. The demand for frozen semen in Indonesia is very high. Kumari *et al*. (2018) stated that detailed knowledge about maintenance behavior of stud bulls was essential for practicing better management conditions in the farm to support the AI industry demand. The process of produce frozen semen is influenced by technical and managerial factors. Technical factors affecting the quality of frozen semen are bull quality, fresh semen quality, dilution, feed quality provided and technology of production. Qualified frozen semen should come from a superior bull. The Bull Breeding Soundness Evaluation (BBSE) is a practice for evaluating bulls prior to use in a breeding season (Penitente-Filho *et al*., 2018).

Bull's fertility becomes a very important factor in AI activities. An AI Center should be able to preserve bulls fertility to produce qualified frozen semen. The evaluation of volume, motility and concentration of semen in the frozen semen production process are the factors affecting the quantity and quality of the frozen semen that can be produced. AI Centre has quality control protocols thresholds for certain sperm quality variables, so that substandard ejaculates were discarded before freezing (Morrel *et al.*, 2017). Evaluation of quantity and quality of semen are influenced by genetic and economic value of frozen semen. The possible relationship between various semen characteristics and fertility is one of some particular importance (Stalhammar *et al*., 1989). Holstein bull fertility has an important influence on reproductive efficiency and genetic quality improvement. Knowledge of factors affecting semen production is important (Fuerst-Waltl *et al*., 2006).

The total numbers of frozen semen produced per collections are affected by the volume, motility and concentration of the fresh semen (Toelihere, 1993). The process of frozen semen production was repeated continuously over the lifetime production of each bull. The concept of the number of repetitions of traits occurred many times over the course of its life and shows the positive phenotypic correlation between present performance and future performance in an individual or repeatability (Hardjosubroto, 1994). Calculating of repeatability on semen traits affecting the production of frozen semen was useful for early selection of bulls as frozen semen producer. The volumes of fresh semen, motility, concentration and number of frozen semen per collection have correlation among them. The objective of this research was to analysis the repeatability and phenotypic correlation among semen quality traits that affecting the frozen semen production from Holstein bull in Indonesia.

Materials and Methods

Data were originated from two national AI center in Indonesia. The value of repeatability of Holstein bull's frozen semen production were calculated based on data of frozen semen production of 15.699 records from 44 Holstein bulls at SAIC with 2-9 years old bulls and based on data of frozen semen production of 8.935 records from 39 Holstein bulls at LAIC with 1-9 years old bulls; in which they were used as frozen semen producer period from 2008 to 2016. Data of the AI centers had to be analyzed separately due to different management and location. The statistical model for calculating repeatability was as follows:

$$Y_{ijk} = \mu + \alpha_i + \beta_{j(i)} + \varepsilon_{(ij)k}$$

i = 1,2,3,..,a j = 1,2,3,..b k=1,2,3,..c

Y_{ijk} = The kth observation, the jth age factor and the ith bull factor
μ = overall mean
α_i = effect of ith bull
$\beta_{j(i)}$ = effect of jth age factor of ith bull
$\varepsilon_{(ij)k}$ = effect of error

Repeatability estimation for semen traits was calculated by intraclass correlation method (Kurnianto, 2012):

$$r_i = \frac{\sigma_b^2}{(\sigma_b^2 + \sigma_w^2)}$$

r_i = The repeatability value of the ith traits of semen quality (volume, motility, concentration, semen production per collection)
${\sigma^2}_b$ = Variance between individuals
${\sigma^2}_w$ = Variance between appearances within individuals

The phenotypic correlation analysis used was a linear correlation analysis according to Saefuddin *et al*. (2009) which illustrate the relationship between two variables measured by the formula as follows:

$$r_x = \frac{\sum_{i=1}^{n}(x_i - \bar{x})(y_i - \bar{y})}{\sqrt{\sum_{i=1}^{n}(x_i - \bar{x})^2}\ \sqrt{\sum_{i=1}^{n}(y_i - \bar{y})^2}}$$

x = x variable
y = y variable
n = total of observation
r = correlation coefficient

Result and Discussion

The use of statistical value of repeatability and correlation on the semen quality traits were effective in the selection process of frozen semen production. Semen evaluation is an important component of the Bull Breeding Soundness Evaluation (BBSE) when performed appropriately (Chenoweth and McPherson, 2016). Despite there exist no reliable tests for evaluating semen quality and predicting bull fertility (Kaya and Memili, 2016).

The traits determining number of production in the frozen semen process were the volume, motility and concentration of fresh semen (Arifiantini, 2012). The volume of fresh semen is one of the earliest standards in evaluating the quality of semen that can be seen directly on collection tube scale. Average of total semen volume of Holstein bull per age at two National AI centers are presented in Table 1. The volume of semen increases continuously with the age of bulls until 7 years old at two AI center and relatively constant until age of 9 years old. Increased volume with age was according to previous studies (Taylor *et al*., 1985; Mathevon *et al*., 1998), which stated that the volume of fresh semen increased up to bulls aged 7 years old and relatively constant until bulls aged 10 years. Semen volume increased with increasing bull age, because the size of the testes and tubuliseminiferi grows larger, so it influence the increasing in sperm cell production. The lowest of semen volume was obtained in groups of bulls <2 years

Table 1. Average of total semen volume of Holstein bull per age at two national AI center

Singosari AI Centre				Lembang AI Centre			
Age	Mean	Std. Dev	N	Age	Mean	Std. Dev	N
1	-	-	-	1	5.40	1.70	95
2	6.18	1.73	1469	2	5.95	2.00	1139
3	6.86	1.84	2279	3	7.39	2.14	1186
4	7.41	2.02	3141	4	7.70	2.32	1908
5	7.72	2.21	2951	5	8.31	2.38	1319
6	8.09	2.27	2215	6	8.20	2.45	1143
7	8.41	2.30	1364	7	8.31	2.57	994
8	8.03	2.16	1278	8	8.49	2.28	741
9	7.95	2.26	1002	9	8.59	2.87	410
Total	7.54	2.19	15699	Total	7.74	2.47	8935

old. The volume of semen reached the top at bull 7 years old (8.41±2.30 ml) at SAIC and 9 years old (8.59±2.87) at LAIC. The effect of age on volume was in accordance with the report of Paldusova *et al*. (2014) stated that the semen volume showed optimal results in the bulls age >5 years and showed the lowest semen volume in age <2 years old. This result was different from the study of Fuerst-Waltl *et al*. (2006) which stated that the highest semen volume was achieved in 6 years old bull in Simmental in Austria. The average semen volume obtained was 7.54±2.19 ml at SAIC and 7.74±2.47 at LAIC. According to Karoui *et al*. (2011), the handling bull factor of the collection affects the semen volume.

Average of total semen motility of Holstein bull at two AI Centre is presented in Table 2. Total average of motility was 66.44±0.1% ranged from 64.91% to 68.59% at SAIC and 65.27±0.14% ranged from 62.74% to 67.38% at LAIC. The motility value of the results of this study was still in the normal range in the statement of Garner and Hafez (2000), which stated that the motility of semen ranged from 40 to 70%. The assessment of semen motility was influenced by the subjectivity of the laboratory technicians who examined it.

Assessment of the concentration of semen was used to determine amount of diluent to be added to the frozen semen production process. Concentration of semen increases at age 1-3 years, and then decreases with increasing age at two AI center (Table 3). Effect of age on semen concentration was accordance with Mathevon *et al*. (1998) which stated that there was an increase in the concentration of semen up to the age 20-22 months then decreases with increasing age. The lowest semen concentration (949.89±448.5 x 10^6) was obtained in bulls aged <2 years old at LAIC. The highest semen concentration (1,330.73±521.5 x 10^6) was obtained in bulls aged 2 years old at SAIC and (1,291.30±427.2 x 10^6) was obtained in bulls aged 3 years old at LAIC.

The average number of frozen semen per collection is presented in Table 4. The highest average production of frozen semen was achieved in 7 years old bulls at SAIC (420±194 dose) and 8 years old bulls at LAIC (387±150 dose), because of >7 years old bull having the highest average volume of fresh semen, the concentration and motility of fresh semen was relatively high and stable at >7 years old bull. The amount of semen volume, concentration and motility were factors determining the quality and amount of frozen semen produced. The average of frozen semen production per collection at two AI center increased at age of 1-3 years old and was relatively stable at 3-9 years old bull. The average numbers of frozen semen per collection were 354±178 doses at SAIC and 321±139 doses at LAIC. Factors affecting semen production in bull sires must be considered in the insemination industry because they affect the productivity of bulls (Snoj *et al*., 2013).

Identifying the genetic basis of bull reproductive traits that influence male and female

Table 2. Average of total semen motility of Holstein bull at two AI Centre

Singosari AI Center				Lembang AI Center			
Age	Mean (%)	Std. Dev	N	Age	Mean	Std. Dev	N
1	-	-	-	1	62.74	0.17	95
2	64.91	0.11	1469	2	64.31	0.14	1135
3	67.13	0.09	2279	3	67.38	0.13	1183
4	67.97	0.09	3141	4	66.42	0.13	1906
5	64.88	0.12	2952	5	65.76	0.13	1316
6	66.57	0.09	2216	6	62.99	0.15	1132
7	68.59	0.08	1364	7	65.24	0.13	992
8	64.92	0.12	1278	8	64.56	0.15	741
9	65.69	0.11	1002	9	63.18	0.17	409
Total	66.44	0.10	15701	Total	65.27	0.14	8909

Table 3. Average of total semen concentration of Holstein bull at two AI Centre

Singosari AI Center				Lembang AI Center			
Age	Mean	Std. Dev	N	Age	Mean	Std. Dev	N
1	-	-	-	1	949.89	448.46	95
2	1330.73	521.50	1469	2	1192.50	452.41	1139
3	1301.16	526.63	2279	3	1291.30	427.18	1186
4	1220.77	505.80	3141	4	1259.10	435.89	1908
5	1139.20	483.61	2952	5	1215.70	347.57	1319
6	1147.03	472.48	2216	6	1059.60	309.97	1143
7	1213.51	440.15	1364	7	1085.90	358.29	994
8	1019.80	457.00	1278	8	1125.60	434.07	741
9	1023.35	501.45	1002	9	1216.60	540.07	410

fertility, and using this information for selection, would improve herd fertility (Thundathil *et al*., 2016). The repeatability values of LAIC for volume, motility, fresh semen concentration and frozen semen production were 0.60; 0.54; 0.37 and 0.47, respectively. While repeatability values of SAIC for volume, motility, fresh semen concentration and frozen semen production were 0.54; 0.30; 0.43 and 0.29, respectively. The value of repeatability was categorized as high, except for semen production at SAIC were moderate (Kurnianto, 2012). The repeatability value of semen traits at Lembang and Singosari AI center is presented in Table 5. The value of repeatability obtained was accordance with the results of research of Chandler *et al*. (1985) in which the value of repeatability ranged from 0.31 to 0.44 and with report of Stalhammar *et al*. (1989) that repeatability for the quality of semen ranged from 0.5 to 0.6. Variance "between of bulls" component (σ_s^2) was higher than that of age and interaction of age "within bulls" will produce a high degree of repeatability (>0.3), but if the variance of "between of bulls" was lower compared to other variance, then it will produce low to moderate repeatability (<0.3). Variance value "between of bulls" showed that the diversity for traits values of frozen semen as a result of different bulls abilities. Calculating the value of repeatability on volume, concentration, motility and frozen semen production can be used in predicting the reproduction performance selection of bulls as frozen semen producer early. The value of repeatability based on previous research for fresh semen volume ranged from 0.22 (Sarakul *et al*., 2018) to 0.64 (Mathevon *et al*., 1998), for fresh semen concentrations from 0.42 (Taylor *et al*., 1985) to 0.64 (Mathevon *et al*., 1998) and for fresh semen motility from 0.17 (Taylor *et al*., 1985) to 0.54 (Stalhammar *et al*., 1989). The difference in repeatability value was due to different estimation methods, population number, age and number of different collection at the time of measurement. Estimation of repeatability values showed the correlation of measurements in the same bulls during their productive lives that were affected by genetic and environmental factors. The high repeatability value can be used as one of the criteria for selection of semen viability from the beginning on Holstein's bull used as frozen semen producers as long as the permanent environmental conditions can be controlled in order to produce repeatability and selection accurately.

Phenotypic correlation among semen quality traits showed positive and negative values. Phenotypic correlations among semen quality traits are presented in Table 6. There was significant (P<0.05) phenotypic correlation between volume and concentration which had value -0.135. Higher volume of semen would cause a lower number of spermatozoa per ml of semen. The correlation value was similar to report of Karoui *et al*. (2011), which stated that the correlation of volume with concentrations was -0.13. The result was different from report of Gredler *et al*. (2007), which stated that the correlation between volume and concentration was positive even though its value was very small, closed to 0 on Austrian Simmental semen. Semen consists of seminal plasma and spermatozoa, so that the higher volume of semen would increase seminal plasma volume and reduced the number of spermatozoa per ml of semen. Furthermore, Taylor *et al.* (1985) stated that bulls were more collected have a greater volume and number of sperm than bulls were rarely collected; even though the proportion of the increases was

Table 4. The average number of frozen semen per collection of Holstein bull at two AI Centre

Singosari AI Center				Lembang AI Center			
Age	Mean	Std. Dev	N	Age	Mean	Std. Dev	N
1	-	-	-	1	203	96.54	63
2	319	172.33	960	2	272	114.85	742
3	355	180.12	1804	3	330	135.85	910
4	359	175.24	2672	4	330	145.24	1478
5	351	158.06	2157	5	313	125.77	1058
6	341	166.89	1805	6	294	123.65	838
7	420	194.22	1149	7	326	143.19	793
8	350	202.46	969	8	387	150.08	558
9	326	195.82	804	9	376	154.87	291
Total	354	178.86	12320	Total	321	139.21	6731

Table 5. Repeatability for semen traits

Semen traits	Lembang AI Center				Singosari AI center			
	σ_s^2	σ_u^2	σ_w^2	r_i	σ_s^2	σ_u^2	σ_w^2	r_i
Volume	1.31	0.30	0.58	0.60	1.85	1.15	0.43	0.54
Motility	0.0035	0.0003	0.0027	0.54	0.0009	0.0010	0.0011	0.30
Concentration	28404.24	29770.34	19069.7	0.37	34192.07	15473.42	29969.24	0.43
Semen production	3947.45	804.57	3671.87	0.47	3681.14	3029.43	6095.48	0.29

Table 6. Estimated value correlation on volume, motility, concentration of fresh semen and number of Holstein's frozen semen production

Semen Traits	Volume	Motility	Concentration	Frozen semen
Volume	1	0.015	-0.135	0.41
Motility	-	1	-	0.36
Concentration	-	0.427	1	0.58

insignificant. The phenotypic correlation between semen concentrations with motility was 0.427 and highly significant (P<0.01). The result was lower than report of Karoi *et al*. (2011) that value of phenotypic correlation was 0.87, but greater than the report of Everett and Bean (1982) that was 0.107. The difference in values could be attributed to a subjective semen motility assessment process, differences in the number of data observations and technologies used in calculating semen concentrations. The phenotypic correlation between volume and semen motility was 0.015 and insignificant (P=0.775). The amount of frozen semen produced per collection was influenced by the concentration, volume and motility of fresh semen (Arifiantini, 2012). The phenotypic correlation value between volume, motility and fresh semen concentration with the amount of semen produced per collections were 0.41, 0.36, and 0.58 (P<0.01). The largest value of phenotypic correlation was 0.58 between the concentration and the number of frozen semen produced per collections; that showed concentration was the most influence factor the number of frozen semen produced. Taylor *et al*. (1985) stated that there are several factors influencing the phenotypic correlation value between the semen traits were the difference between the number of bull's collection and the interaction between permanent genetic, age and environment factors.

Semen characteristics could be used to predict the fertility, when the correlation between a combination of traits and the real fertility were assessed. Bull breeding soundness evaluation (BBSE) was easily performed, repeatable, and associated with the subsequent fertility of the bull (Hancock *et al*., 2016) The effectiveness of the selection of Holstein's frozen semen producing determined by the value of repeatability and the phenotypic correlation between semen traits. Januskauskas *et al*. (2000) stated a significant relationship between the traits of semen quality, especially semen motility, with bull's fertility to result pregnancy in females from AI activity. In fact, the data of cow pregnancy on AI activity per bulls was difficult to collect in wide quantity and accuracy. This research can helps AI center to optimize semen production by selection, controlling permanent environment factor and bulls health in producing qualified frozen semen.

Conclusions

The volume of semen increasing continuously in parallel with the age of bulls until 7 years old and was relatively constant until the age of 9 years old. Semen concentration increased at age of 1-3 years old. The highest of average frozen semen production was achieved at >7 years old. The value of repeatability semen traits were categorized as high. Concentration was the most influence factor affected number of frozen semen that could be produced. The effectiveness of the selection of Holstein's frozen semen producing determined by repeatability and the phenotypic correlation among semen traits.

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