Effect of Age and Bulls on Fresh Semen Quality and Frozen Semen Production of Holstein Bulls in Indonesia

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Effect of Age and Bulls on Fresh Semen Quality and Frozen Semen Production of Holstein Bulls in Indonesia

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Abstract

Artificial Insemination is a compatible method of reproduction in an effort to increase dairy productivity. Artificial Insemination Center as a producer of frozen semen was required to maximize bulls in producing high quality frozen semen optimally. The purpose of this research was to determine effect of age and bulls on fresh semen quality and frozen semen production of Holstein bulls in Indonesia. The research was conducted at Lembang and Singosari AI Centers. The material used were 24.634 data of qualified fresh semen and frozen semen production from 81 Holstein Bulls aged 1-9 years that used as frozen semen producer in period of 2008 to 2016. The variables observed in this research were data of age of bulls, fresh semen volume (mL); sperm motility (%); mass movement; concentrations (million/mL) and frozen semen doses at each production at age of bulls. Nested design was applied to obtain and analyze data. Results showed that Age and bulls have significant effect (P<0,01) to volume, mass, motility, concentration and frozen semen production. Increasing the age of bulls resulted in increase semen volume until 7-year-old, while semen concentration decreased from 3 years old with increasing age. Frozen semen production, mass movement and motility shown the same relative value on 3-9 years old except on 1 to 2 years old had increase. Bulls would produce frozen semen optimally on 3- 9 years old. Indeed, with knowledge of this factor, AI Centre might adapt management of AI bulls to improve semen production.

1. Introduction

Increased demand for milk was stimulating farmers to increase productivity of dairy cattle in order to supply market needs. Efforts to import dairy cows continuously will lead to dependency and depletion of foreign exchange, therefore another alternative was required by providing a source of high quality frozen semen that will be used in artificial insemination (AI). An increase in dairy productivity can be achieved through improved reproduction systems and genetic improvement.

Artificial Insemination is a compatible method of reproduction in an effort to increase dairy productivity. The success of AI implementation in the field was influenced by the quality of the frozen semen used [1]. Semen quality could be assessed based on its fresh semen characteristics through macroscopic or microscopic examination [2]. High quality frozen semen from a superior bull has a role in improving fertility and reproduction efficiency. Holstein bulls are "biological machine" having a productive age in producing frozen semen. Productive age is the period during the bull could maximize production optimally and efficiently in producing frozen semen. Lestari et al. [3] showed that age has a significant effect on fresh semen volume so that the age factor is very important in determining the quality and quantity of semen and can be used as part of the selection criteria for bull as frozen semen production.

Artificial insemination centre as a producer of frozen semen must be maximize bulls that capable to produce semen with good fertility so factors affecting semen production are very important

such as effect of age [4]. Ismaya [5] stated that the quality of semen was influenced by several factor, those were: age of bulls, genetics, temperature and season, collecting and feeding frequency. The results of [6] showed that age had a significant effect on the volume of Simmental bulls in Austria.

2. Materials and Methods

2.1. Material and location

The research was conducted at Lembang and Singosari AI Centre. The material used were 24.634 data of qualified fresh semen and frozen semen production from 81 Holstein bulls aged 1-9 years that used as frozen semen producer in period of 2008 to 2016. The variables observed in this research were data age of bulls, fresh semen volume (mL); semen motility (%); mass movement; concentrations (million/mL) and frozen semen doses at each production age of bulls.

2.2. Statistical analysis

Data were analyzed with nested design, to calculate the value of production efficiency. Statistical model:

$$Y_{ijk} = \mu + A_i + B_{j(i)} + s_{(ij)k}$$

Y_{ijkl} = the k^{th} observation of j^{th} age factor i^{th} and bulls factor
μ = overall mean
A_i = effect of i^{th} bull factor
$B_{j(i)}$ = effect of j^{th} age factor of i^{th} bull factor
$s_{(ij)k}$ = effect error

3. Results and Discussion

Level of significance for bulls and bull's age class at 2 National AI Centre in Indonesia are presented in Table 1. As data of the 2 National AI centre had to be analyzed separately due to different data recording, comparison between centres are not feasible. Bulls and bull's age were highly significant for all traits in both AI centre.

Table 1. Level significance for age and bull effect at 2 national AI Centre

	Vol		Mass		Mot		Con		FS	
	1	2	1	2	1	2	1	2	1	2
Bulls	**	**	**	**	**	**	**	**	**	**
Bull's age class	**	**	**	**	**	**	**	**	**	**

1, Lembang AI Centre; 2, Singosari AI Centre; Vol, ejaculate volume; Mass, mass movement; Mot, motility; Con, sperm concentration; FS, total frozen semen.
**, ($P<0.01$)

a. Effect of Age and Bulls on Semen Volume of Holstein Bulls

The volume of fresh semen is one of the earliest standards in evaluating the quality of semen that can be seen directly on the tube scale of the collection. Data on the volume of fresh semen at 2 National AI center are presented in Table 2. The average volume of semen in Lembang and Singosari AI center had increased with age of bulls. The highest volumes of bulls achieved on 7 years old at Singosari and

9 years old in Lembang were in agreement with [6] stated that the highest ejaculate volume were achieved by bull being older than 72 months.

The results of the variance analysis showed a very significant difference ($P < 0.01$) between the fresh semen volume of Holstein bulls at different ages (Table 1). It was accordance with research [6] which stated that the age had a significant influence on the volume of semen Simmental bulls in Austria.

Table 2. Volume of Fresh Semen at 2 National AI center

Singosari AI Centre			Lembang AI Centre		
Age	Mean	Noumber of Collection	Age	Mean	Noumber of Collection
1	-	-	1	5.41±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±2.29	741
9	7.95±2.26	1002	9	8.59±2.88	410
Total	7.54±2.19	15699	Total	7.74±2.47	8935

Total average of semen volume of Holstein bull per age at 2 National AI centers can be seen in Table 3. The volume of semen continues increased with the age of bulls until 7 years old and was relatively constant at the age of 9 years old. It were in accordance with the study of [5] which stated that the volume of fresh semen continuously enhanced with increasing age, but decreased for older bulls. Increasing volume with age was in accordance with previous studies [7] and [8] stated volume of fresh semen increased up to bulls aged 7 years old and relative constant until bulls aged 10 years. However, unlike [9], the highest semen volume is obtained in bulls aged 9 year-old, but not significant in bulls aged 5-9 years. The volume increased with the age of bulls due to the growing size of the larger testes where tubuliseminiferi growth up so that influenced increased production of spermatozoa cells [9]. The lowest semen volume in the bull group were <2 years old and increase with age. The volume of semen reach the top at bull's age 7 years (8.368 ± 2.41 mL) and after that time gradually decreased in older bulls. It was in accordance with the results of research [10] 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. It was different from the study of [6] which obtained top semen volume at 6 year old of Simmental bulls in Austria.

b. Effect of Age and Bulls on Semen Mass Movement of Holstein Bull

The mass movement of semen is the simultaneous movement of spermatozoa that causes waves [11]. Semen mass movement was used as a selection in frozen semen production, where fresh semen must be has a minimum value 2+ to produce as frozen semen. Data on the mass movement of Holstein bulls per ages at Singosari AI center are showed in Table 4. Mass movement 2+ had highest percentage (97.6%) and mass movement 3+ had lowest percentage (0.5%). The highest percent value of 2+ mass movements were obtained in bulls aged 6 years (99%), while 1+ mass movement were mostly found in bull's age 2 year (3%). The percentage value of mass movement 1+ under 10% illustrates as good

bulls management and semen handling, where the percentage of fresh cement unprocessed was less than 10%.

Table 3. Total average of semen volume of Holstein bull

Age	Mean	Noumber of collection
1	5.40±1.70	95
2	6.08±1.85	2608
3	7.04±1.96	3465
4	7.52±2.14	5049
5	7.90±2.28	4270
6	8.12±2.33	3358
7	8.36±2.41	2358
8	8.19±2.21	2019
9	8.13±2.47	1412
Total	7.61±2.29	24.634

Table 4. Semen mass movement at Singosari AI Center

		Ages								
		2	3	4	5	6	7	8	9	Total
Mass	1 Count	44	32	48	78	19	15	30	28	294
	% within age	3.0	1.4	1.5	2.6	0.9	1.1	2.4	2.8	1.9
	2 Count	1415	2234	3062	2857	2189	1312	1246	969	15284
	% within age	96.4	98.1	98.1	96.9	99.0	97.1	97.6	96.7	97.6
	3 Count	9	12	11	12	4	24	0	5	77
	% within age	0.6	0.5	0.4	0.4	0.2	1.8	0.0	0.5	0.5
Total	Count	1468	2278	3121	2947	2212	1351	1276	1002	15655
	% within age	100	100	100	100	100	100	100	100	100

Table 5. Semen Mass Movement at Lembang AI Center

			Ages									
			1	2	3	4	5	6	7	8	9	Total
Mass	1	Count	32	383	269	414	256	288	198	182	118	2140
		% within age	33.7	33.7	22.7	21.7	19.5	25.4	20	24.6	28.9	24
	2	Count	61	744	896	1474	1056	843	792	554	284	6704
		% within age	64.2	65.5	75.7	77.3	80.2	74.5	79.9	74.8	69.4	75.2
	3	Count	2	9	18	18	4	1	1	5	7	65
		% within age	2.1	0.8	1.5	0.9	0.3	0.1	0.1	0.7	1.7	0.7
Total		Count	95	1136	1183	1906	1316	1132	991	741	409	8909
		% within age	100	100	100	100	100	100	100	100	100	100

Based on data are presented in Table 5, mass movement 2+ in Lembang AI center had highest percentage (75.2%) and mass movement 3+ had the lowest percentage (0.7%). The highest percentage of mass movements 2+ were obtained in 5 year olds (80.2%), while mass movements 1+ were highest in bulls aged 1 and 2 years (33.7%). Hafez [12] stated that the normal mass movement of cement was 2+ to 3+. The percentage of mass movement 1+ more than 10% indicated bulls management and cement handling were non optimal, where the percentage of fresh cement than unprocessed greater than 10%.

Table 6. Average value mass movement of Holstein bulls

Age	Mean	Noumber of Collection
1	1.68±0.51	95
2	1.85±0.42	2604
3	1.92±0.30	3461
4	1.91±0.30	5027
5	1.92±0.30	4263
6	1.91±0.29	3344
7	1.92±0.31	2342
8	1.90±0.31	2017
9	1.90±0.32	1411
Total	1.91±0.32	24564

c. Effect of Age and Bulls on Semen Motility of Holstein Bull

Testing sperm motility is one of the important parameters that can be used as the basic information about the ability of spermatozoa in fertilization [14]. Data sperm motility of Holstein bulls at different ages are showed in Table 7. The value of semen motility at 2 national AI center had the lowest score (62.74%) in bull aged <2 years old at Lembang AI center and had the highest score (68.59%) in bulls aged 7 year old at Singosari AI center. The value of motility was increased with age. That was according to the study of [9] which stated that the increasing age had a significant effect on the sperm motility.

The result of variance analysis showed a significant difference (P <0.01) motility of Holstein bulls at different ages (Table 1). This was subject to the research by [6] that stated age gave a real effect on the individual motility.

Total average motility semen of Holstein bull at 2 AI Center was presented in Table 8. Result of research got mean motility (66.02%) with range (62.74%-67.38%), consistent with [15] fresh semen had an average motility (48.7% -79.9). The motility value of the results of this study was still within the normal range in the opinion of [12] which stated that the motility of semen ranges from 40-75%.

Table 7. Semen Motility at 2 National AI center

Singosari AI Center			Lembang AI Center		
Age	Mean[1]	Noumber of collection	Age	Mean[1]	Noumber of Collection
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

1. Motility (%)

Table 8. Total average motility semen of Holstein bull

Age	Mean (%)	Noumber of Collection
1	62.74±0.18	95
2	64.65±0.12	2604
3	67.21±0.10	3462
4	67.38±0.11	5047
5	65.15±0.12	4268
6	65.36±0.11	3348
7	67.18±0.10	2356
8	64.79±0.13	2019
9	64.96±0.13	1411
Total	66.02±0.12	24.610

d. Effect of Age and Bulls on Semen Concentration of Holstein Bull

Evaluation of semen concentration was used to determine the amount of diluent to be added in the frozen semen production process. The results of semen concentration of Holstein bulls at different ages are showed at Table 9. The highest semen concentration at Singosari AI center was obtained at bull aged 2 years old (1330.73 x 10^6), concentration decrease with increasing age, according to the study of [6] which stated that the concentration decreased with increasing age and reach optimum value for bull aged 18-20 months. While the highest cement concentration at Lembang AI center was obtained at bull aged 3 year old (1291,30 x 10^6) then decreased with increasing age.

Table 9. Descriptive Statistic for Semen Concentration

Singosari AI Center			Lembang AI Center		
Age	Mean [1]	Noumber of Collection	Age	Mean [1]	Noumber of Collection
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

1. Semen Concentration ($x10^6$/mL)

The result of variance analysis showed that age had significant effect (P <0,01) to the semen concentration of Holstein bulls (Table 1). This is accordance with the study of [6] which stated that age had a significant effect (P <0.01) on semen concentration. Concentration of semen increases at age 1-3 years, and then decreases with age (Table 10). That was in accordance with [8] which stated that there was an increase in the concentration of semen up to the age 20-22 months then decreases with age. The lowest semen concentration (949.89 ± 448.4 x 10^6) was obtained in bulls aged <2 years old and the highest (1,297.8 ± 4.94 x 10^6) was obtained in bulls aged 3 year olds. This was different from [16] study which stated that bull's age 4 years old would have the highest concentration of semen.

Table 10. Semen concentration averages of Holstein bulls at different ages

Ages	Mean (10^6/mL)	Noumber of Collection
1	949.89±448.46	95
2	1270.40±497.18	2608
3	1297.80±494.80	3465
4	1235.30±480.89	5049
5	1162.80±447.41	4271
6	1117.30±426.20	3359
7	1159.70±412.41	2358
8	1058.60±451.50	2019
9	1079.50±520.23	1412
Total	1187.40±471.88	24.636

e. Effect of Age and Bulls on Frozen Semen Production of Holstein Bull

Total frozen semen results in the frozen semen production process were influenced by quality of fresh semen, diluent and production process. The average data on total frozen semen produced by the Holstein bulls at different ages are showed in Table 11. The highest average of frozen semen production was achieved at bulls aged 7 years (420 doses) in Singosari and bull aged 8 years (387 doses) in Lembang. This is accordance with [9] who stated that cement production increased significantly in bulls aged over 60 months. Total average production of frozen semen at singosari was higher (354 doses) than Lembang (321 doses).

Table 11. Descriptive Statistic for Frozen Semen Production

Singosari AI Centre			Lembang AI Centre		
Age	Mean	Noumber of Collection	Age	Mean	Noumber of Collection
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

The results on Table 12 showed that the highest average frozen semen production at bull aged 7 years old (382 ± 181.14 straw) that was caused by bull aged 7 years old had highest average volume of fresh semen with other variable (semen concentration and motility) were quite large. This is accordance with [17] noted an increase in the amount of semen with age of the animal. The amount of semen volume, concentration and motility are factor that determines the quality and amount of frozen semen produced. The average of frozen semen production per collection increased at bull aged 1-3 years and reached a plateau at 3-9 years of age.

The determination of productive age was calculated based on the percentage of frozen semen production per collection per age that multiplied by average of frozen semen production per age are showed at Table 13. Result calculation of optimal production age shows that bulls age 3 to 9 year old had value of frozen semen productivity above the average total productivity, while 1-2 years old bulls had value of frozen semen productivity below total average, so it could be determined that bulls will be produce frozen semen optimally on 3-9 years old. Taylor et al. [7] observed increasing sperm production until 84 month of age and started to decrease after 112 month of age, while [18] reported that sperm production declined after 84 months of age. Indeed, with knowledge of this factor, AI Centre might adapt management of AI bulls to improve semen production.

Table 12. The average production of Holstein frozen semen per collection per ages

Age	Frozen semen/collection/age	
	Mean	Coeficient of Variation (%)
1	203±96.54	47.56
2	298±151.80	50.94
3	347±166.98	48.12
4	349±165.73	47.49
5	339±149.29	44.04
6	326±156.03	47.86
7	382±181.14	47.42
8	364±185.82	51.05
9	340±187.05	55.01
Total	343±166.67	48.59

Table 13. Calculation productive age of bull

Age	Noumber of Collection	Processing/collection/age		Frozen semen /collection/age	Productivity◆
		Noumber of	%		
1	95	63	66.32	203	134
2	2608	1702	65.26	298	195
3	3465	2714	78.33	347	271
4	5049	4150	82.19	349	287
5	4271	3215	75.28	339	255
6	3359	2629	78.27	326	255
7	2358	1942	82.36	382	315
8	2019	1526	75.58	364	275
9	1412	1095	77.55	340	263
AVG	2737.33	2115.11	75.68	327	250

◆, Productivity = (frozen semen/collection/age) x (% processing/collection/age)

4. Conclusion

The conclusions that can be drawn from this research are Age and bulls had significant effect (P<0,01) to semen volume, semen mass movement, semen motility, semen concentration and frozen semen production. Increasing the age of bulls result increasing semen volume until the 7 year-old, while the semen concentration decreases from 3 years old with increasing age. Frozen semen production, mass movement and motility show the same relative value in bull's age 3-9 years old except on bull age 1-2 years old has increase. Bulls would produce frozen semen optimally on 3-9 years old. Indeed, with knowledge of this factor, AI Centre might adapt management of AI bulls to improve semen production.

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