

PRODUCTIVITY AND FRESH SEMEN CHARACTERISTICS OF SIMMENTAL BULLS OF DIFFERENT AGES

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ABSTRACT

This study was aimed at determining the effects of age on the productivity and characteristics of fresh semen of Simmental bulls in Indonesia. A total of 1071 pieces of data from the collected samples of semen and production characteristics of the bulls from four age groups (two years old, yo), four yo, ≥ 10 yo with high semen rejection (≥ 10 HR), and ≥ 10 yo with low semen rejection (≥ 10 LR) were used in this study to evaluate the productivity and characteristics of fresh semen (semen volume, sperm concentration, total sperm concentration, total sperm motility, individual motility, and mass movement). The results showed that the pre-freezing and post-freezing semen rejection rate of the ≥ 10 HR group was higher ($P < 0.05$) than the other groups. The four year old group had twice the percentage of ejaculation frequency per collection ($P < 0.05$) compared to the other groups (the highest). Semen volume, sperm concentration, and total sperm concentration significantly increased ($P < 0.05$) until the age of four, and then decreased ($P < 0.05$) in the ≥ 10 yo groups. The ≥ 10 HR group had a significantly lower volume and total sperm concentration ($P < 0.05$) than the ≥ 10 LR group. All of the characteristics of the fresh semen, including total sperm motility, individual motility, and mass movement in the ≥ 10 HR group were lower ($P < 0.05$) than those in the other groups. In conclusion, age differences of the bulls did affect the ejaculation frequency, semen volume, sperm concentration, and total sperm concentration of each collection. The semen rejection rate of older bulls directly affects all of the characteristics and productivity of the fresh semen of Simmental bulls.

Key words: age, bull, characteristic, fresh semen, productivity

ABSTRAK

Penelitian ini bertujuan mengkaji pengaruh umur terhadap produktivitas pejantan dan karakteristik semen segar sapi Simmental di Indonesia. Sebanyak total 1071 data koleksi dan produksi semen dari empat kelompok umur (dua tahun, empat tahun, ≥ 10 tahun dengan tingkat penolakan (rejection) semen tinggi (≥ 10 HR) dan ≥ 10 tahun dengan tingkat rejection semen rendah (≥ 10 LR)) digunakan dalam penelitian ini untuk dianalisis produktivitas dan karakteristik semen segar antara lain volume semen, konsentrasi sperma, total konsentrasi sperma, persentasi total motilitas, gerakan individu, dan gerakan massa. Hasil penelitian menunjukkan bahwa tingkat pre-freezing dan post-freezing semen rejection pada kelompok umur ≥ 10 HR terlihat paling tinggi ($P < 0,05$) dibandingkan dengan kelompok lainnya. Kelompok umur empat tahun memiliki persentasi frekuensi ejakulasi sebanyak dua kali per koleksi paling tinggi ($P < 0,05$) dibandingkan kelompok lainnya. Volume semen, konsentrasi sperma dan total konsentrasi sperma meningkat secara signifikan ($P < 0,05$) hingga umur empat tahun, kemudian menurun secara signifikan ($P < 0,05$) pada kedua kelompok umur ≥ 10 tahun. Kelompok umur ≥ 10 HR tahun memiliki volume dan konsentrasi total sperma paling rendah secara signifikan ($P < 0,05$) dibandingkan kelompok umur ≥ 10 LR. Persentasi total motilitas, gerakan individu, dan gerakan massa pada kelompok umur ≥ 10 HR terlihat paling rendah secara signifikan ($P < 0,05$) dibandingkan dengan umur lainnya. Disimpulkan bahwa perbedaan umur pejantan sapi Simmental dapat memengaruhi frekuensi ejakulasi per koleksi, volume semen, konsentrasi sperma, dan total konsentrasi sperma. Tingkat penolakan semen pada sapi berumur tua secara langsung berpengaruh terhadap produktivitas dan semua parameter karakteristik semen segar sapi Simmental.

Kata kunci: umur, sapi, karakteristik, semen segar, produktivitas

INTRODUCTION

Artificial insemination (AI) is an assisted reproductive technology that has been widely used with the aim of increasing the reproductive efficiency and genetic quality of livestock. One of the exotic breeds of cattle in Indonesia that is widely used for AI activities is the Simmental cattle. These cattle are often used for cross-breeding with various local cattle breeds in Indonesia, such as Bali cattle, Madura cattle, and Ongole crossbreed cattle. Cattle have a large muscle mass, high body weight gain, and a greater body weight overall when compared to local cattle (Sutarno and Setyawan 2016).

Bulls have an important role in improving the quality of the offspring produced. The contribution of bulls to the quality of their offspring is genetically inherited through spermatozoa. Therefore, semen

quality is an important factor in regards to increasing the success of AI (Suyadi *et al.* 2020). The quality of a bull's semen is influenced by several factors, including age (Balic *et al.* 2012; Prihatin *et al.* 2017; Komariah *et al.* 2020), breed, environment, and nutrition (Prihatin *et al.* 2017; Komariah *et al.* 2020). Bulls typically produce the optimal quality of semen at the age of 3-4 years (Saeed *et al.* 1989). Hallap *et al.* (2004) stated that the reproductive capacity of bulls seems to decrease gradually after reaching the age of 3-4 years. However, the fact is that there are still many bulls over ten years old whose semen is still used for AI activities.

Changes in semen quality are in line with increasing age and are associated with physiological changes such as an increased body mass (Balic *et al.* 2012) which also simultaneously affects the development of the testes and accessory glands of bulls (Murphy *et al.* 2018). Changes in semen quality are also influenced by

changes in the activity of the hypothalamus-pituitary-testicular axis in regards to producing reproductive hormones (Johnson *et al.* 1990; Murphy *et al.* 2018). This can affect the process of spermatogenesis in the testes which has an impact on the productivity of bulls. Rashid *et al.* (2015) stated that the age of bulls can affect productivity due to changes in sperm production capacity, storage capacity in the cauda epididymis, and the number of sperm cells ejaculated. Schenk (2018) explained that at productive age the testes develop faster after puberty on into adulthood because the seminiferous tubules become longer with a wider diameter so that spermatogenesis is more optimal. In addition, the cauda epididymis has the capacity to store large amounts of sperm and is able to expand and contract maximally to accommodate the number of sperm cells to be ejaculated (Rashid *et al.* 2015; Schenk 2018). The productivity of bulls is not only measured by the volume of semen produced but also by the quality of other aspects of the semen itself.

Evaluation of the quality of fresh semen from bulls is usually carried out by macroscopic and microscopic examination (Komariah *et al.* 2020). This evaluation is important in order to describe early the process of spermatogenesis, accessory gland secretion patterns, and functional competence of spermatozoa (Srivastava and Pande 2017). Therefore, evaluation of the quality of fresh semen is used as the basis for decision-making in the cryopreservation process (Nagata *et al.* 2019; Peris-Frau *et al.* 2020). Research on Bali cattle (Nugraha *et al.* 2019), Ongol crossbreed cattle (Suyadi *et al.* 2020), Sahiwal cattle (Ahmad *et al.* 2011; Bhakat *et al.* 2011), and Frieswal cattle (Mandal *et al.* 2009) showed that the quality of fresh semen such as motility, sperm concentration, and semen volume was influenced by differences in age. Although Simmental cattle in Indonesia have been used for a long time for AI, there is still little information about the relationship between age and the quality of fresh semen and its production capacity. Therefore, this study was conducted to examine the effects of age on the productivity and characteristics of fresh semen from Simmental bulls. This information will assist policymakers and users in formulating new policies for the breeding and culling of bulls that will be used for semen production.

MATERIALS AND METHODS

Research Design

Production records and ejaculate data was divided into four groups, namely a) two-year-old bulls ($n = 3$), b) four years old bulls ($n = 3$), c) ≥ 10 years old bulls with a high semen rejection rate (≥ 10 HR; $n = 3$), and d) ≥ 10 years old bulls with low a semen rejection rate (≥ 10 LR; $n = 3$).

Data Collection

Production records and ejaculation data was obtained from 12 healthy Simmental bulls from the Lembang Artificial Insemination Center, Bandung,

West Java during January-December 2020. The bulls were housed individually, maintained, and fed according to the applicable rearing system. Production record data shows the number of semen collections and the production processes from fresh semen to frozen semen. Fresh semen quality data analyzed included volume, mass movement, motility, individual movement, and concentration. Semen collection was performed twice a week using the artificial vaginal method and was carried out by experienced technicians. Semen volume was measured using a scale tube, sperm motility was evaluated under a light microscope with a magnification of 400 \times , and sperm concentration was evaluated using a spectrophotometer. All secondary data was then tabulated using Microsoft Excel 2010.

Data Analysis

This study used a completely randomized design (CRD) with four treatments, the age of the bulls. Quantitative data in this study was analyzed using one-way analysis of variance (ANOVA) and continued with Duncan's Multiple Range Test (DMRT). All data was displayed in the form of mean \pm standard deviation (SD).

RESULTS AND DISCUSSION

Productivity of Simmental Bulls of Different Ages

The productivity of Simmental bulls of various ages in this study was evaluated based on the rate of semen rejection and the frequency of ejaculation for each semen collection. The total number of collections for all age groups was 1071 times with the highest number of semen collections being found in the four-year age group and the least in the ≥ 10 HR age group (Table 1). Based on Table 1, the rate of pre-freezing and post-freezing semen rejection in the ≥ 10 HR age group was the highest ($P < 0.05$) compared to the other groups. Furthermore, the ≥ 10 HR age group had a significantly lower amount of processed semen ($P < 0.05$) compared to the other groups. The high pre-freezing semen rejection was due to the fact that the fresh semen did not meet the minimum standards for frozen semen processing, meaning that the fresh semen motility was less than 70%, individual sperm movement was less than 2, and mass movement was less than ++. The minimum standard for processing frozen bull semen was in accordance with SNI 4869-1:2017 concerning frozen semen for cattle and Permentan number 10/Permentan/PK.210/3/2016 concerning the supply and distribution of frozen semen for ruminants. Thippeswamy *et al.* (2014) also found that the high pre-freezing semen rejection was due to the bull producing poor quality semen, which led to the semen being unfit for freezing. Meanwhile, post-freezing semen rejection was carried out because the quality of post-thawing semen did not meet the minimum standards according to SNI 4869-1:2017 regarding frozen bovine semen, namely having minimum post-thawing motility of 40%, minimum individual movement of 2, minimum number of spermatozoa cells 25 million per dose and a

minimum recovery rate of 50%. High post-freezing semen rejection is also associated with lower sperm viability after freezing (freezability) due to a high incidence of cryodamage (Rego *et al.* 2016; Nagata *et al.* 2019). Cryodamage is caused by high oxidative stress in the spermatozoa (Nagata *et al.* 2019; Peris-Frau *et al.* 2020) due to an imbalance between reactive oxygen species (ROS) production and antioxidants (Gunes *et al.* 2016; Peris-Frau *et al.* 2020). The high oxidative stress in this study was thought to be due to several factors, including the cryopreservation process, aging of the bulls, and differences in protein expression in the semen. The process from cryopreservation to thawing causes an imbalance in the production of ROS and antioxidants which can lead to cellular changes, such as membrane destabilization, loss of cholesterol, and decreased fluidity, which causes spermatozoa to become more susceptible to cryodamage (Castro *et al.* 2016; Amin *et al.* 2018; Nagata *et al.* 2019; Peris-Frau *et al.* 2020). Furthermore, aging of bulls is associated with an increased production of ROS that can initiate oxidative stress (Weir and Robaire 2007; Aitken 2020) as well as induce lipid peroxidation (Gunes *et al.* 2016; Ahmed *et al.* 2018). Meanwhile, the plasma membrane of spermatozoa contains large amounts of polyunsaturated fatty acids (PUFA) which are very susceptible to oxidation (Castro *et al.* 2016; Amin *et al.* 2018). Therefore, this situation can exacerbate the occurrence of cryodamage in spermatozoa. Research by

Rego *et al.* (2016) adds that there is an expression of several semen proteins in abundance which is associated with sperm resistance to cryodamage in the group of bulls with high freezability.

The ejaculation frequency of each semen collection can also describe the semen production ability of bulls (Figure 1). Based on the figure, the 4-year-old group had the highest percentage of semen ejaculation frequency, twice per collection, which was significantly higher ($P < 0.05$) compared to the other groups. Although the percentage of semen ejaculation frequency was once per collection in the four-year age group, it was the lowest ($P < 0.05$) compared to the other groups. The frequency of ejaculation is related to the capacity of the testes and epididymis of the bulls (Prihatin *et al.* 2017). Schenk (2018) states that the age of the bulls greatly affects the capacity of the testes and sperm storage in the epididymis. The capacity of the testes can be evaluated through the testes measurement approach (Ahmad *et al.* 2011; Vijetha *et al.* 2014; Sankhi *et al.* 2019). Measurement of scrotal circumference is an accurate method for estimating testes size (Schenk 2018). Scrotum circumference is positively correlated with daily sperm production capacity, number of sperm ejaculated, and sperm storages in the epididymis (Rashid *et al.* 2015; Sankhi *et al.* 2019). Research by Vijetha *et al.* (2014) showed that Nallore cattle aged 3-5 years had the largest scrotal circumference and had the highest sperm production

Table 1. Rejection rate of Simmental bull semen different age

Bull's age (Yo)	Total of semen collection	Semen rejection		Semen processed
		Pre-freezing	Post-freezing	
2	249	6 (2.32±1.73 ^a)	15 (7.00±7.64 ^a)	228 (90.68±7.53 ^a)
4	274	19 (6.67±5.59 ^a)	18 (6.58±1.82 ^a)	237 (86.74±4.39 ^a)
≥ 10 HR	243	43 (17.13±3.83 ^b)	51 (18.65±11.18 ^b)	149 (64.21±14.42 ^b)
≥ 10 LR	251	13 (5.12±0.84 ^a)	25 (9.92±8.54 ^a)	213 (84.97±8.96 ^a)
P-Value		0.008	0.046	0.006

^{a, b}Different superscripts in the same column indicate a significantly different $P < 0.05$. Yo= Years old; ≥ 10 HR= ≥ 10 years old with high semen rejection rate; ≥ 10 LR= ≥ 10 years old with low semen rejection rate

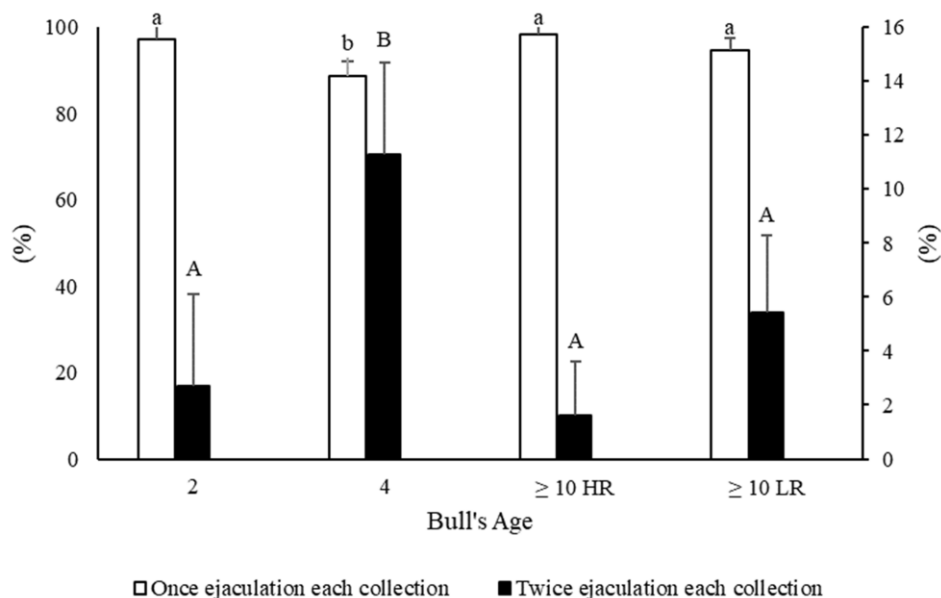


Figure 1. Percentage of frequency of ejaculation each semen collection in Simmental bull different ages. The different superscripts (a dan b) in group of once ejaculation each collection and superscripts (A dan B) in group of twice ejaculation each collection indicate a significantly different $P < 0.05$

capacity compared to other ages. Rashid *et al.* 2015 also added that large scrotal circumference in adult bulls affects daily sperm production and semen volume. This is because the cauda epididymis has the ability to expand and contract to accommodate more or less sperm to be ejaculated. In adult bulls, the cauda epididymis has the capacity to store sperm in the epididymis in large numbers. The increased capacity of the cauda epididymis can encourage the testes to produce more sperm, affecting daily sperm production and ejaculation frequency (Schenk 2018).

Characteristics of Fresh Semen of Simmental Bulls with Different Ages

Table 2 shows that semen volume, sperm concentration, and total sperm concentration increased significantly ($P<0.05$) until the age of four years, then decreased significantly ($P<0.05$) in both groups of ≥ 10 years of age. The ≥ 10 HR years old groups had significantly lower total sperm volume and concentration ($P<0.05$) than the ≥ 10 LR age group, although both groups had the same concentration in each reservoir. This is closely related to changes in testicular morphology due to increasing age (Jiang *et al.* 2013; Bhanmeechao *et al.* 2018). Tesi *et al.* (2020) showed that histologically the area of the epithelium and seminiferous tubules will increase from peripubertal to adult age and then decrease due to the aging of bulls. Other studies have also shown that aging in bulls leads to increased fibrosis of testicular interstitial connective tissue (Bhanmeechao *et al.* 2018), degeneration of germ cells, and seminiferous tubules (Peters *et al.* 2000; Jiang *et al.* 2013;

Bhanmeechao *et al.* 2018). These changes cause a decrease in spermatogenic cells, Leydig cells, and Sertoli cells (Jiang *et al.* 2013; Bhanmeechao *et al.* 2018). Furthermore, changes at the cellular level of the testes can lead to changes in reproductive hormones in males. Johnson *et al.* (1990) showed that an increase in the age of bulls was associated with increased levels of follicle-stimulating hormone (FSH) and decreased serum testosterone (Johnson *et al.* 1990). These changes directly caused a disruption of the processes of spermiogenesis and spermatogenesis due to aging (Jiang *et al.* 2013; Bhanmeechao *et al.* 2018). This situation resulted in changes in the semen quality such as semen volume, sperm concentration, and total sperm count (Johnson *et al.* 1990; Tesi *et al.* 2020). The results of this study are in line with the research of Bhakat *et al.* (2011) which showed that the semen volume and spermatozoa concentration were highest ($P<0.05$) in Sahiwal cattle aged 3-4 years and then decreased after that age. Furthermore, research by Mandal *et al.* (2009) in Frieswal cattle also showed an increase in semen volume and spermatozoa concentration from young age (<30 months) to peak at adult age (31-70 months).

Another parameter of sperm quality that is very important to evaluate is motility. Sperm motility is a parameter that is often used as an indicator to predict fertility (Li *et al.* 2014; Magdanz *et al.* 2019). This is because motility tends to use the greatest amount of energy, which is around 70% of the total ATP consumption in spermatozoa (Bohnsack and Halangk 1986; Tourmente *et al.* 2015). In addition, motility also indicates the functional ability of sperm cells which

Table 2. Volume, sperm concentration and total sperm concentration of Simmental bull different ages

Bull's age (Yo)	Vol (mL)	SpC ($\times 10^6$ /mL)	TspC ($\times 10^6$)
2	4.88 \pm 1.75 ^a	1084.47 \pm 236.06 ^{ab}	5444.62 \pm 219.18 ^a
4	7.31 \pm 2.69 ^b	1121.64 \pm 340.10 ^a	8554.66 \pm 335.33 ^b
≥ 10 HR	5.97 \pm 1.87 ^c	1022.66 \pm 256.83 ^c	6230.22 \pm 209.44 ^c
≥ 10 LR	6.49 \pm 1.97 ^d	1062.05 \pm 285.38 ^{bc}	7104.84 \pm 263.09 ^d
P-Value	0.000	0.001	0.000

^{a, b, c, d}Different superscripts in the same column indicates a significantly different $P<0.05$. Yo= Years old; ≥ 10 HR= ≥ 10 years old with high semen rejection rate; ≥ 10 LR= ≥ 10 years old with low semen rejection rate; Vol= Volume; SpC= Sperm concentrations; TspC= Total sperm concentration

Table 3. Sperm mass motility of Simmental bull different ages

Bull's age (Yo)	Number of semen collection	Sperm mass motility			
		Strong (+++)	Medium (++)	Weak (+)	None
2	249	0 (0.00 \pm 0.00)	244 (98.04 \pm 1.12 ^a)	4 (1.60 \pm 0.53 ^a)	1 (0.36 \pm 0.62)
4	274	0 (0.00 \pm 0.00)	262 (95.64 \pm 2.00 ^a)	12 (4.36 \pm 2.00 ^a)	0 (0.00 \pm 0.00)
≥ 10 HR	243	0 (0.00 \pm 0.00)	205 (84.64 \pm 2.21 ^b)	35 (14.25 \pm 0.71 ^b)	3 (1.11 \pm 1.92)
≥ 10 LR	251	0 (0.00 \pm 0.00)	239 (95.30 \pm 2.91 ^a)	12 (4.70 \pm 2.91 ^a)	0 (0.00 \pm 0.00)
P-Value	-	-	0.000	0.000	0.526

^{a, b}Different superscripts in the same column indicate a significantly different $P<0.05$. Yo= Years old; ≥ 10 HR= ≥ 10 years old with high semen rejection rate; ≥ 10 LR= ≥ 10 years old with low semen rejection rate

Table 4. Sperm total motility and individual motility of Simmental bull different ages

Bull's age (Yo)	TM (%)	IM
2	70.12 \pm 5.59 ^a	1.97 \pm 0.19 ^a
4	69.33 \pm 6.70 ^a	1.93 \pm 0.26 ^a
≥ 10 HR	66.26 \pm 11.37 ^b	1.82 \pm 0.39 ^b
≥ 10 LR	69.42 \pm 5.79 ^a	1.95 \pm 0.23 ^a
P-Value	0.000	0.000

^{a, b, c, d}Different superscripts in the same column indicates a significantly different $P<0.05$. Yo: Years old; ≥ 10 HR= ≥ 10 years old with high semen rejection rate; ≥ 10 LR= ≥ 10 years old with low semen rejection rate; TM= Total motility; IM= Individual motility

play an important role in successful fertilization (Li *et al.* 2014). In this study, motility parameters were evaluated based on three approaches, namely total motility, individual motility, and mass motility. Total motility describes the ratio of motile sperm compared to total sperm (Kathiravan *et al.* 2011). Individual motility are used to assess if the sperm can move forward, backward, coil in place or not move at all. Thus, the progressive motility of individual spermatozoa can be used as an important vitality index in the spermatozoa population (Susilawati 2011). Furthermore, the mass motility of spermatozoa describes the movement of spermatozoa simultaneously to form a mass wave, so that the thicker the mass wave formed, the better the sperm quality and the more highly correlated with fertility (David *et al.* 2015). The mass motility of spermatozoa is shown in Table 3, and the total motility and individual motility in more detail are shown in Table 4. The tables show that the ≥ 10 HR age groups have the lowest percentage of total motility, individual motility, medium mass motility, and weak mass motility ($P < 0.05$) when compared with other age groups. This is presumably because this group has the highest level of oxidative stress compared to the other groups. Aitken (2020) stated that oxidative stress is caused by overproduction of ROS due to aging in bulls. ROS overproduction can be caused by several things, including aging of bulls, stress, leukocytosis, radiation and toxins (Agarwal *et al.* 2014). Oxidative stress can cause lipid peroxidation (Gunes *et al.* 2016; Dutta *et al.* 2019) which can impair plasma membrane permeability and mitochondrial dysfunction (Amaral *et al.* 2013; Dutta *et al.* 2019; Aitken, 2020). Therefore, it causes a decrease in ATP production and impairs ATP efflux (Dutta *et al.* 2019), whereas ATP produced by the inner mitochondrial membrane will be transferred to the microtubules to regulate the motility of spermatozoa (Oberoi *et al.* 2014). Thus, the incidence of oxidative stress is closely related to decreased sperm motility (Agarwal *et al.* 2014; Dutta *et al.* 2019).

CONCLUSION

The age of bulls affects the frequency of ejaculation per collection, semen volume, sperm concentration, and total sperm concentration. The rate of semen rejection in older bulls directly affects the productivity and characteristics of the fresh semen of Simmental bull. It is necessary to conduct further research on the quality of frozen semen in order to obtain an overview of the freezability of each age group of bulls, and evaluate the level of fertility in vivo and in vitro.

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