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To cite this article: R D Hapsari et al 2018 *IOP Conf. Ser.: Earth Environ. Sci.* 142 012007

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Age effect on post freezing sperm viability of Bali cattle (*Bos javanicus*)

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Abstract. Post freezing sperm viability is one of factors which determine artificial insemination success. In the other side, bull’s or sire age influences the semen quality through sperm membrane constituent. It is known that freezing process change the sperm membrane during the processing stage. This research aims to know the effect of sire age on post freezing sperm viability of Bali cattle. The samples were collected in Singosari Artificial Insemination Centre, Malang, East Java, Indonesia on September - November 2016. Eight Bali cattle (4 and 7 y.o, 4 heads in each group) were used as semen source. Semen was collected using artificial vagina, 10 times spanning for 5 weeks (2 times per week, interval 3 and 4 days) in a row. The samples were then evaluated at fresh, chill and frozen stage. Fresh semen was diluted in Tris-citrate-egg yolk 20% (v/v) followed with chilling and freezing. semen qualities were observed as sperm % motility (MOT), % live sperm using eosin-nigrosine staining (EOS) and % sperm membrane integrity using hypoosmotic swelling test (HOS). Variable comparisons between age groups were done using t-test. On the average, 4 y.o bulls showed higher semen quality at fresh, chill and frozen compared to 7 y.o in MOT (68.00±6.39 vs 65.9±7.62; 56.40±3.71 vs 54.33±5.83; 44.25±3.52 vs 40.40±7.06), EOS (72.08±6.63 vs 71.82±7.38; 57.81±3.83 vs 57.41±6.32; 53.16 ±8.41 vs 46.49±9.13) and HOS (60.85±13.91 vs 54.84±13.43; 53.16 ±8.41 vs 46.49±9.13; 44.6±9.39 vs 33.8±10.70) respectively. Statistical analysis results showed that age was significantly (P<0.05) affecting HOS at chill stage and MOT and HOS at frozen. In conclusion, younger Bali cattle (4 y.o) have more viable post freezing sperm compared to the older ones (7 y.o).

1. Introduction

Bali cattle is an Indonesian native which has many advantages, like well adapted to the tropics [1] and high carcass percentage and high fertility [2]. Fertility of Bali cattle is higher compared to Europe cattle or another tropical cattle, that is 82–86% [3]. To spread that genetic potential, artificial insemination (AI) is the efficient way compared to other reproductive technology. AI is a first generation reproductive biotechnology that has made a profound contribution to the genetic improvement[4]. To be able to use as superior bull, the fertility of Bali cattle need to be tested through observation of libido ability and semen characteristics both macroscopic and microscopic. In term of semen quality, it is influenced by breed, reproductive health status, technical skills, age and bull genetic constitution [5].

Sperm viability will determine the success of AI. Low sperm viability will decrease the achievement of conception rates. Bull’s age is very important factor to determining the sperm...
viability. Previous study shows that bulls at age 2 to 7 y.o are able to produce high quality semen with high conception rates compared to older [6]. Moreover, in young bull (less than 2 y. o; at puberty), more abnormal sperm produced with account to low fertilization rate[7]. During frozen semen production, temperature change the quality and structure of sperm membrane. It is reported that these condition modify lipids and alter functional state of membrane[8], therefore more or less 50% of sperm will die during freezing process [9]. Based on the previous description, this study aimed to know the effect of age on post freezing sperm viability of Bali cattle.

2. Materials and Methods

2.1. Semen samples
This study was conducted in Singosari Artificial Insemination Centre, Malang, East Java, Indonesia during September - November 2016. Eight Bali cattle (4 and 7 y.o, 4 head in each group) were used as semen source. Semen was collected using artificial vagina, 10 times spanning for 5 weeks (2× per week, interval 3 and 4 days) in a row. Fresh semen was diluted in Tris- citrate -egg yolk 20% (v/v) followed with chilling and freezing. The samples were then evaluated at fresh, chill and frozen stage.

2.2. Semen quality
Semen qualities were observed as:

2.2.1. Sperm motility (MOT) It can be defined as the percentage of sperm which moving in straight forward direction. Sperms were observed by placing the drop of samples semen on the slide glass followed by observation under microscope (40× magnificient). Sperm motility was scored 0–100%.

2.2.2. Livesperm (EOS) Live sperms were evaluated with differential staining technique using eosin-nigrosine dye according to the previous protocol [10]. The death sperm will absorb the dye and the observation was done in 200 sperms followed with percentage calculation as follows:

\[ \% \text{EOS} = \frac{\text{live spermatozoa}}{\text{total spermatozoa}} \times 100\% \]

2.2.3. Sperm membrane integrity(HOS) The sperm membrane integrity was evaluated using hypoosmotic swelling test. Two microliter of semen sample were mixed with 20 µL hypoosmotic solution (0.90 g D-fructose and 0.490 g Trisodium citrate in 100 ml aquadest with osmolarity 150 m osmoles), followed with homogenization and incubation at 37°C for 30 minutes. The incubated solution were then evaluate with differential staining technique using eosin-nigrosine dye. Sperms with intact membrane will show swelling or coiling tail [10]. Moreover, the percentage of intact sperm membrane was calculated using following formula:

\[ \% \text{HOS} = \frac{\text{intact spermatozoa}}{\text{total spermatozoa}} \times 100\% \]

2.2.4. Statistical analysis Semen quality comparison between age groups was done using t-test in this study. All the statistical analysis and graph presentation were done using R language and statistical environment.

3. Result and discussion
On the average, 4 y.o bulls shows higher semen quality at fresh, chill and frozen stage compare to 7 y.o (Table 1). The result showed that age is significantly affecting HOS at chill stage and MOT and HOS at frozen stage. This result is similar with the previous study [11] which shows that age gave significant influence to the MOT. The MOT and HOS were affected by bull age because of their viability. Earlier study reported that genetic, age, breed and individual will influence the sperm
viability with the thermal shock during the thawing[12]. Moreover, 4 y.o have better sperm viability in the thermal shock compared to 7 y.o. In the younger bull and has higher semen quality, show lower decrease from fresh to chill or frozen stage (Table 2 and Figure 1).

Table 1. Mean and standard deviation of semen quality based on bull’s age

| Bull age (y.o) | MOT (%) | EOS (%) | HOS (%) |
|---------------|---------|---------|---------|
| Fresh stage   |         |         |         |
| 4             | 68.00±6.39 | 72.08±6.63 | 60.85±13.91 |
| 7             | 65.90±7.62 | 71.82±7.38 | 54.84±13.43 |
| Chill stage   |         |         |         |
| 4             | 56.40±3.71 | 57.81±3.83 | 53.16±8.41⁹ |
| 7             | 54.33±5.83 | 57.41±6.32 | 46.49±9.13⁹ |
| Frozen stage  |         |         |         |
| 4             | 44.25±3.52⁹ | 39.47±9.57 | 44.60±9.39⁹ |
| 7             | 40.40±7.06⁹ | 35.16±11.74 | 33.80±10.70⁹ |

⁹Different superscripts within same row differ significantly (p<0.05)

Table 2. Decrease of semen quality based on bull’s age

| Variables | Processing stage | Bull age |
|-----------|------------------|---------|
|           |                  | 4 y.o   | 7 y.o   |
| MOT (%)   | Fresh            | 68.00±6.39 | 65.90±7.62 |
|           | Chill            | 56.40±3.71 | 54.33±5.83 |
|           | Frozen           | 44.25±3.52⁹ | 40.40±7.06⁹ |
| EOS (%)   | Fresh            | 72.08±6.63 | 71.82±7.38 |
|           | Chill            | 57.81±3.83 | 57.41±6.32 |
|           | Frozen           | 39.47±9.57 | 35.16±11.74 |
| HOS (%)   | Fresh            | 60.85±13.91 | 54.84±13.43 |
|           | Chill            | 53.16±8.41 | 46.49±9.13 |
|           | Frozen           | 44.6±9.39 | 33.8±10.70 |

Figure 1. The decrease of semen quality during semen processing

The decrease of semen quality was suggested by the damage of sperm membrane. Cryopreservation process in frozen semen production will induces the damaged of sperm membrane [13]. The cryopreservation has number of potential stress that damaging plasma membrane such as the temperature variation, osmotic and toxic stress, and the formation of ice in the extracellular environment[8]. The damage sperm membrane commonly occurs in the midpiece where mitochondria are located. As reported mitochondria are wrapped around midpiece to provide energy for tail movement [14], therefore any damage of mitochondria resulting low sperm motility.
4. Conclusion
In this study, it is concluded that younger Bali cattle has more viable sperm compared to the older ones at post freezing stage. That result might be accounted by the condition of sperm membrane in 4 y.o bulls that better compared to 7 y.o.

References
[1] Talib C, Entwistle K, Siregar A, Budiarti-Turner S and Lindsay D 2002 Survey of population and production dynamics of bali cattle and existing breeding programs in Indonesia Strategies to Improve Bali Cattle in Eastern Indonesia (ACIAR Proceedings) pp 2–9
[2] Bamualim A and Wirdahayati R B 2002 Nutrition and management strategies to improve Bali cattle productivity in Nusa Tenggara Strategies to Improve Bali Cattle in Eastern Indonesia (ACIAR Proceedings) pp 17–22
[3] Gunawan, Pamungkas D and Affandhy L 1998 Sapi Bali (Yogyakarta: Kanisius)
[4] Lemma A and Shemsu T 2015 Effect of age and breed on semen quality and breeding soundness evaluation of pre-service young bulls J. Reprod. Infertil. 6 35–40
[5] Bhakat M, Mohanty T K, Raina V S, Gupta A K, Khan H M, Mahapatra R K and Sarkar M 2011 Effect of age and season on semen quality parameters in Sahiwal bulls Trop. Anim. Health Prod. 43 1161–8
[6] Yulianto P and Saparinto C 2014 Beternak Sapi Limousin (Jakarta: Penebar Swadaya)
[7] Hafez E S E 1993 Reproduction in Farm Animals (Maryland: Lippincott Wiliams and Wilkins)
[8] Watson P F 2000 The causes of reduced fertility with cryopreserved semen Anim. Reprod. Sci. 481–92
[9] Lessard C, Parent S, Leclerc P, Bailey J L and Sullivan R 2000 Cryopreservation alters the levels of the bull sperm surface protein P25b J. Androl. 21 700–7
[10] Shukla M K 2011 Applied Veterinary Andrology and Frozen Semen Technology (New Delhi, India: New India Publishing Agency)
[11] Aminasari P D 2009 Pengaruh Umur Pejantan terhadap Kualitas Semen Beku Sapi Limousin (Malang: Universitas Brawijaya)
[12] Chenoweth P J 2005 Genetic sperm defects Theriogenology 64 457–68
[13] Januskauskas A, Johannisson A and Rodriguez-Martinez H 2003 Subtle membrane changes in cryopreserved bull semen in relation with sperm viability, chromatin structure, and field fertility Theriogenology 60 743–58
[14] Connell M O, Mcclure N and Lewis S E M 2002 The effects of cryopreservation on sperm morphology, motility and mitochondrial function Hum. Reprod. 17 704–9