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CHARACTER MOTILITY OF LIQUID SEMEN ON ONGOLE CROSSBREED (PO), BALI AND MADURA BULLS WITH DIFFERENT DILUENTS AT COLD STORAGE

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Abstract - Motility of spermatozoa is one of the parameters used to determine the fertility status of the bulls. The purpose of this study is to measure sperm motility in liquid semen using epididymis Caudal Plasma-2 (CEP-2) diluent on Ongole crossbreed (PO), Bali and Madura bulls. This study used 6 bulls consisted of 2 PO bulls, 2 Bali bulls and 2 Madura bulls which is located in Beef Cattle Research Institute, Grati Pasuruan. Semen collection was done by using an artificial vagina and then proceed with the analysis of fresh semen and liquid semen prosessed. Observation of liquid semen quality conducted until the 5th day. Examination of liquid semen motility used Computerized Assisted Semen Analysis (CASA). Parameters measured include: fresh semen quality (volume, pH, colour, consistency, concentration, mass movement, viability, motility, totally motile sperms (TSM), and abnormalities of spermatozoa) and liquid semen quality (motility, progressive motility, velocity curve is linear (VCL), velocity straight linear (VSL), velocity average pathway (VAP), linearity (LIN), straighness (STR), wobble (WOB), hyperactivity (H), amplitude of lateral head (ALH) and beat cross frequency (BCF) . Analysis data used Minitab 17 and SPSS 16. Motility parameters of liquid semen with CEP-2 diluent on PO, Bali and Madura bulls during cold storage showed almost the same value. Bali cattle and PO indicate motility and progressive motility higher than Madura bulls. Velocity (VCL, VSL and VAP) of Bali and Madura bulls higher than PO. Value of LIN and ALH were relatively stable during cold storage. Value of STR fluctuates in every breed and WOB value indicates a downward trend in all breeds during cold storage. Value of BCF on PO bulls was higher than Bali and Madura. The usage of CEP-2 diluent in the processing of liquid semen can support sperm motility on PO, Bali and Madura bulls until the 5th days of cold storage.

INTRODUCTION

Bull fertility is determined by several factors including scrotal circumference and semen quality. The main parameters of semen quality which is used to determine bulls fertility are motility and morphology of spermatozoa (Parker *et al*.,1999). Motility of spermatozoa is a movement of spermatozoa from one spot to another spot. This parameter plays an important role in predicting the ability of sperm swimsto reach and penetrate the egg. At this time, semen diluents have been developed both herbal and chemical. One of the chemical diluent that is widely used recently is Caudal Epididimis Plasma (CEP-2). Excellence of CEP-2 including the compositions consisted of ions and osmolarity which resembles to the composition of seminal plasma in the epididymis. This condition can support the quality of sperm, motility and membrane integrity of spermatozoa (Ducha *et al*., 2012; Arif *et al*., 2013; Indriani et al, 2013; Purwoistri *et al*., 2013). Previous research of Ducha *et al*., (2012) indicate that CEP-2 diluent combined with 20% egg yolk can protect membrane integrity of sperm and maintain motility until the 8th day (44.45%). This diluent has an important role

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to support the fertilization capability of spermatozoa during storage (Zaenuri *et al*., 2012). Firdausi *et al*., (2014) stated that the composition of the CEP-2 and yolk 10% can minimize abnormality spermatozoa and maintain the membrane integrity.

Motility assesment can be done visually or using CASA. Motility assesment using CASA provides a more objective assessment, specific and automatically to assess morphology, concentration and motility of spermatozoa (Didion, 2008; Verstegen *et al*., 2001). Computerized Assisted Semen Analysis (CASA) is a method of image analysis (movement pattern) using phase contrast microscopy and computerized systems. Lange-Consiglio *et al*., (2013) stated that CASA is a very useful instrument for characterizing the motility of spermatozoa because they are objective, independent (of interpretation and analysts) and to identify the specific movement of spermatozoa.

The purpose of this study is to measure sperm motility of liquid semen with CEP-2 diluent ondifferent breeds of bull by using CASA.

MATERIALS AND METHODS

Site and Time of Research

The study was conducted in cages and Laboratory Animal Reproduction Beef Cattle Research, Grati Pasuruan. The duration of the study was for 3 months, starting in November 2016- January 2017.

Materials

This research used 6 bulls aged 4-5 years old, consisted of 2 bulls/breed (PO, Bali and Madura).

Methods

Collecting fresh semen was done by using artificial vagina until 10 repetitions. Cement storage is done by using artificial vagina. Fresh semen with motility >70%is suitable for processing into liquid semen. Spermatozoa concentrations were calculated using a haemocytometer. The standard concentration of liquid semen is 100 million / ml. The formula of calculation is as follows:

$$V1 \times M1 = V2 \times M2$$

Description

V1: Volume of semen
M1: Concentration of semen
V2: Volume of liquid semen (semen + diluent)
M2: Concentration of liquid semen(100 million/mL)

The result of mixing of semen and diluent (liquid semen) is placed on glass reaction tube and placed on backer glass without water jacket. Glass reaction tube covered with plastic wrap. The liquid semen is stored in the refrigerator at 3-5 ^{o}C. Evaluation of the motility of liquid semen up to the 5th day using the CASA system is Sperm Class Analyzer (SCA) version 5.2 Microptic, Barcelona, Spain. Frame rate (FR) CASA is 25 fps.

The composition of CEP-2 diluent including: NaCl 15 mmol/L KCl 7 mmol/L, $CaCl_2(H_2O)_2$ 3 mmol /L, $MgCl_2(H_2O)6$ 4 mmol/L, $NaHCO_3$ 11.9 mmol /L, NaH_2PO_4 8 mmol /L, KH_2PO_4 20 mmol/L, Fructose 55 mmol/L, Sorbitol 1 g/L, Tris 133.7 mmol/L, gentamicin-S 0.05 g/L, 10% egg yolk and egg white 0.4%, citric acid 42 mmol/L.The addition of 0.4% albumin and 10% egg yolk was done at the end of the dilution making step. Centrifuse of diluent for supernatant is taken and ready to be used as liquid semen diluent.

Fresh Semen Examination

a. pH, assessment used pH indicator paper (Susilawati, 2011).
b. Colour, assessment was done subjectively (Susilawati, 2011).
c. Volume, assessment was done by using a scaled tube (Susilawati, 2011).
d. Consistency, assessment of viscosity subjectively by dragging-scale movement of semen in the tube (Susilawati, 2011).
e. Concentration, concentration calculations using haemocytometer (Hafez, 2008; Ax *et al*., 2008).
f. Mass movement, assessment was done subjectively by droping sperm on the slide concave, then examined under microscope magnification 100 x. Level assessment of the mass movement refers to Susilawati (2011).
g. Fresh semen motility assessment was done visually by 2-3 analyst.
h. Totally motile sperm (TSM) is the result of multiplying the sperm concentration, motility and semen volume (Susilawati 2011).
5. Examination of motility with CASA

Starting with a drop of 3-4 µL of liquid semen on glass object that has been warmed at temperature 37 ^{o}C and then covered with a cover glass. Setting a microscope at phase contrast at pH 1 and magnification 10 x 10. Coating the reflector mirror with a green filter (Anonymous, 2016). Setting the diaphragm and light intensity to the color

standards specified by the microscope. Taking picture in 5 fields of view. Results of analysis of each image and its average of 5 fields available in excel form.

Examination of Viability and Abnormalities Spermatozoa

Assesment viability of spermatozoa was done by making smear semen. Dispense 1 drop of semen on the object glass and add 1 drop of stain then mixture. Smear of the mixture on the object glass then fixative with fire. Asssment was done by observing smear wuth a microscope magnification 1.000 times and counted up to 100 sperms. Live spermatozoa observed in transparant colour, dead spermatozoa observed in red colour. The calculation formula was:

$$\frac{\text{Number of live sperm} \times 100\%}{\text{Number of spermatozoa were observed (live and dead)}}$$

Observation of sperm abnormality used a smear semen on object glass as used in viability assesment. Analyze a smear object glass untuil 100 spermatozoa under microscope magnification 1000 times (Ax *et al*., 2008). The calculation formula was:

$$\frac{\text{Number of abnormal spermatozoa} \times 100\%}{\text{Number of spermatozoa were observed (normal and abnormal)}}$$

Parameter of Research

Parameters were measured during the research activities, including:

a. Fresh semen: volume, pH, color, consistency, concentration, mass movement, viability, motility, totally motile sperms and abnormalities of spermatozoa.
b. Liquid semen: motility, progressive motility, sperm hyperactivity, VCL, VAP, VSL, LIN, STR, WOB, BCF, and ALH.

Design of Experiments and Data Analysis

Research experimental design using a completely randomized design (CRD). Analysis of data using Minitab 17 and SPSS 16.

RESULTS AND DISCUSSION

Fresh Semen Quality

Fresh semen quality is the basis of the initial consideration that the semen can be processed into a liquid or frozen semen. Previous research stated that decent fresh semen is processed into frozen semen when motility of fresh semen reached 70% (Sarastina,2002). In this study indicated fresh semen quality data are listed in Table 1.

Different superscripts in the same row indicate significant differences (P <0.05)

Volume and mass movements showed no significantly differences between breeds. Madura bulls semen volume was higher (P <0.05) than PO and Bali. Meanwhile, the mass movement of spermatozoa on Bali and PO are higher (P <0, 05) than Madura bulls. Sperm concentration among breeds already fullfilling standard for concentration bulls, i.e. 200-1800 million/mL (Ax *et al*., 2008). Viability of sperms reached 80%, progressive motility above 50% and spermatozoa abnormalities below 20%

Table 1. Fresh Semen Quality of PO, Bali and Madura Bulls

No.	Fresh Semen Parameters	Breed of Bull		
		PO	Bali	Madura
1.	Volume (mL)	4.7 ± 1.9ab	3.4 ± 0.7a	5.7 ± 0.5b
2.	pH	6.6 ± 0.4	6.6 ± 0.2	6.6 ± 0.5
3.	Colour	cream	cream	cream transparant
4.	Consistency	medium-thick	medium-thick	medium-thick
5.	Concentration (million/mL)	1286.0 ± 230.0	1228.0 ± 265.2	1076.0 ± 127.6
6.	Mass movement	3.0 ± 0.0a	2.6 ± 0.5a	2.0 ± 0.0b
7.	Viability (%)	84.0 ± 6.2	83.6 ± 4.3	85.0 ± 5.3
8.	Progressive motility (%)	71.0 ± 2.2	71.0 ± 2.2	66.0 ± 6.5
9.	TotallyMotile Sperms(million/mL)	4349.0 ± 2139.7	2979.9 ± 947.7	4020.9 ± 758.9
10.	Abnormality (%)	2.2 ±1.5	3.4 ±1.9	4.4 ± 1.8

indicated that semen quality fulfilled standards of bull semen (Ax *et al*., 2008). Some abnormalities spermatozoa were found in this study are primary and secondary abnormality. Primary abnormality including: doubleheadsperms, microchepalic-sperms, pear-shapedspermsand ruffleacrosome. Secondary abnormalities were found including: tailcoiledsperms, absentheadsperms, decapitated sperms.

Sperm Motility of Liquid Semen During Cold Storage

Motility parameters that can be measured using SCA version 5.2, including: motility, progressive motility, VCL, VSL, VAP, LIN, STR, WOB, H, ALH and BCF.

Progressive motility and motility of spermatozoa

Motility and progressive motility of the spermatozoa is a motility parameters level 1 in motility analysis using CASA. Basic classification at CASA motility refers to human standards according to the World Health Organization (WHO), because the animal standard is not yet available. There are four categories of motility in outline, including: fast progressive (VAP> 80 μm/s), slow progressive (80 μm/s> VAP> 25 μm/s), motile non-progressive (VAP <25 μm/s) and immotil (VAP = 0) (Contri *et al*., 2010). Massanyi *et al*., (2008) stated that the spermatozoa motile moving with a speed > 5 μm/s. Meanwhile, a progressive motile spermatozoa moving with a speed > 20 μm/s.

Different superscripts in the same row indicate significant differences (P <0.05)

Based on Table 2, it was known that motility and progressive motility not significantly different between breeds at H0 and H5 of cold storage. The average value of motility and progressive motility spermatozoa on Bali and PO is higher than Madura bulls. This is consistent with the result of research Sarastina (2006) which stated that sperm motility of Bali and PO higher than Madura bulls. During storage, motility and progressive motility of spermatozoa Madura bulls decreased faster. Didion (2008) stated that every breed has a difference of spermatozoa characteristic and fertility. Based on the result of Sholikhan et al (2016) stated that chilled semen at day 5 of storage using CEP-2 diluent reaches a value of motility45.0 + 10.0%, viability 82.9 + 4.0%, abnormalities 4.1 + 1.5% and total motile spermatozoa on day 6 was 41.5 + 6.7 million/mL. While the research results of Da costa et al (2016a) stated that usageof CEP-2 as a diluent at day 5 on PO bulls have motility 51%, viability 80.85%, membrane integrity 77.55%. Continued by Da Costa *et al*. (2016b), stated that using anegg yolk Tris aminomethan in liquid semen on day 1 and 5 days which is used to artificial insemination in PO cows respectively 30 heads reached Conception rate (CR) of 86.66% and 83.33%.

Spermatozoa motility is influenced by many factors, i.e. length of storage and diluent (Zaenuri *et al*., 2012). It is associated with variations in oxygen uptake and metabolic activity in each diluent. Each composition ofCEP-2 diluent has its own role in supporting the quality of spermatozoa during storage, including motility. Fructose and sorbitol in CEP-2 diluent are a energy source and support sperm motility. Albumin and yolk serves as a cryoprotectant which maintain the membrane integrity that also affect to sperm motility (Ducha *et al*., 2012; Arif *et al*., 2013; Indriani *et al*., 2013; Purwoistri *et al*., 2013; Firdausi *et al*., 2014). Some components that serve as a buffer and keep diluent isotonis are: tris, citric acid, NaCl, KCl, $CaCl_2$ $(H2O)_2$,$MgCl_2(H_2O)6$, $NaHCO_3$, NaH_2PO_4, KH_2PO_4. The buffer serves to maintain the PH in order not to decrease due to the pile of lactic acid so that the motility of spermatozoa is maintained (Widjaya, 2011). During cold storage, spermatozoa metabolism continues with side-effects such as reactive oxygen species (ROS) that can damage membrane integrity (Ducha *et al*., 2014).

Spermatozoa motility in all breeds showed a decline be in accordance with the length of storage.

Table 2. Motility and Progressive Motility of Liquid Semen three breeds.

Parameters	Day of Storage	PO	Bali	Madura
Motility	0	96.8±1.9	96.5±1.6	92.3±2.1
	5	79.9±6.8	83.8±8.2	75.3±12.2
Prog. Motility	0	78.5±6.5	83.7±4.8	84.6±3.2
	5	62.1±6.5	61.4±10.6	51.1±10.4

Table 3. VCL, VSL and VAP Liquid Semen of three Breeds.

Parameters	Day of Storage	PO	Bali	Madura
VCL	0	50.2±4.3b	55.0±2.3ab	59.6±8.2a
	5	41.6±6.4	42.0±6.0	45.1±7.2
VSL	0	26.9±5.4b	33.1±4.6a	32.1±4.6ab
	5	21.3±3.7	20.6±3.2	23.0±3.5
VAP	0	35.9±4.6b	42.7±3.5a	41.7±6.0a
	5	25.6±3.1	25.7±4.0	29.2±4.0

Table 4. LIN, STR and WOB Liquid Semen of three Breeds.

Parameters	Day of Storage	PO	Bali	Madura
LIN	0	53.2±8.6	59.6±6.6	54.3±8.0
	5	52.0±10.7	49.1±3.2	51.4±6.0
STR	0	74.5±6.3	76.8±4.4	77.5±8.7
	5	82.7±6.6	80.3±2.7	78.7±2.6
WOB	0	71.1±6.5	77.4±4.6	69.9±3.7
	5	62.2±8.4	61.2±3.4	65.2±6.8

Motility and progressive motility plays an important role in as perm efforts to reachoocyte and penetratezonapellucida. Motility value indicates the state of life and metabolism of spermatozoa. The other role of motility is showing the status of the cell membrane spermatozoa. A motile spermatozoa have an intact membrane cell, whereas immotile spermatozoa has adamaged intact membrane cell (Sarastina, 2006). This is consistent with the results of research Ducha *et al.*, (2014) that there are two main factors that determine the ability of sperm fertilization. The membrane integrity has a major role in forming bonds with the zonapellucida. Meanwhile, the second is sperm motility plays an important fuction to penetrate cumulus and zonapellucida. The variation of value semen results from CASA due to the variation between bulls and their genetic differences among bulls (Farrell *et al*., 1997).

VCL, VAP, VSL

There are three parameters speed of sperm (velocity)that can be measured with SCA version 5.2, including: VCL, VSL and VAP. Each parameter indicates a specific speed of spermatozoa and interrelated between parameters.

Different superscripts in the same row indicate significant differences (P <0.05)

Table 3 showed that the value of VCL, VSL and VAP at H0 significantly different (P <0.05) and not significantly different (P> 0.05) at H5 between breeds.The VCL value on Madura was significantly (P <0.05) higher (59.6%) than PO, but not significantly different (P> 0.05) with the Bali bulls. The VSL value at H0 and H1 are significant differences (P <0.05) in Bali was higher (33.1 μm/s and 30.5 μm/s) than PO (26.9 μm/s and 24.4 μm/s), but not significantly different (P> 0.05) with cattle bulls (32.1 μm/s and 27.0 μm/s). The VAP Value at H0 significantly different (P <0.05) between Bali (42.7 μm/s) than PO (35.9 μm/s), but not significantly different from Madura bulls (41.7 μm/s).

The average value of VCL, VSL and VAP of liquid semen in three breeds declined gradually during at cold storage. The average value of VSL and VAP in Bali higher than Madura and PO bulls. The mean of VSL is highest in Madura than Bali and PO bulls. The results of the study are in accordance with Sarastina (2006) which stated that semen characteristics of Bali bulls resemble Madura bulls. As pointed out earlier that sperm motility and velocity is related to the availability of energy sources in the diluent at a cold storage (sorbitol and fructose).

The VSL value describes the velocity of spermatozoa in one direction / path and used as the main indicator of the progressivity of spermatozoa (Perreault, 2002). Value percentage of motile spermatozoa have a high correlation with VCL and low correlation with VSL. Results of research Holt *et al*., (2007) stated that the average value VSL of bull is 81.4 μm/s. The difference is very far away with this study. However, there were many factors can affected, i.e. breed of bull (Farrell *et al*, 1997).Curve linear velocity (VCL) is a spermatozoa speed along its path. The value VCLis a good

Table 5. Liquid Semen Hyperactivity of three breeds.

Parameters	Day of Storage	PO	Bali	Madura
Hiperactive	0	5.6±2.5	5.2±2.0	7.8±1.0
	5	3.8±2.7ab	8.4±3.9a	2.0±1.1b

Table 6. ALH and BCF Liquid Semen of three Breeds.

Parameters	Day of Storage	PO	Bali	Madura
ALH	0	1.8±0.2	1.9±0.1	2±0.1
	5	1.8±0.3	2.1±0.2	1.7±0.1
BCF	0	11.1±1.6b	10.6±1.3b	13.0±0.8a
	5	12.3±1.0	11.4±1.2	14.1±3.5

indicator for the"vigor"of sperm movement, but does not illustrate the direction of movement of sperm as well as the progression of spermatozoa. Value VSL and VCL is complementary information (Perreault, 2002).Velocity average pathway (VAP) calculated by determining the average length of the path and dividing by the time elapsed to get VAP expressed in μm/s. The value of VAP depends on the average number centroid and the algorithms used. Sperm with relative movement straight on his track and regular, the VAP value similar to VSL than VCL (Perreault, 2002). Tardif *et al*., (1997) stated that media is the main factor contributing to the variations of semen quality at cold storage.

LIN, STR, WOB

An observation results of LIN, STR and WOB of liquid semen during storage are listed in Table 4.

Different superscripts in the same row indicate significant differences (P <0.05)

Based on Table 4, there was no significantly different of LIN, STR and WOB between breeds at H0 and H5 of cold storage. Value of LIN, STR and WOB are motility sperm parameters were derived from the value of other motility parameters, namely VSL, VCL and VAP. TheSTR value is VSL/ VAP multiplied by 100; while LIN is VSL/VCL multiplied by 100 and WOB is VAP/VCL multiplied by 100. The LIN parameter is an indicator of the track straightness curvelinear, STR is an average track straightness spatial and WOB an oscillation measurement of the actual trajectory (Udrayana, 2009). Spermatozoa has categorized as a progressive if STR and LIN is higher than the sperm that swim in a circle (circular swimming pattern).Value of STR and LIN are used as an attempt to identify patterns of swimming spermatozoa, including: spermatozoa hiperactive (vigorous) but it is not progressive and not linear.

Sperm Hyperactivity

Assessment of hyperactivity using a CASA is motility assesment level 2. Some parameters motility level 2 are linear, linearcurve and non-linear (Sarastina, 2006). However, CASA used in this study can measure hyperactivity parameters only. The observation of liquid semen parameters of sperm hyperactivity of three breeds in CEP-2 diluent during cold storage are listed in Table 5.

Different superscripts in the same row indicate significant differences (P <0.05)

Based on Table 5, it is known that sperm hyperactivity significantly different at H5. At H5, values sperm hyperactivity of Bali bulls were significantly different (P <0.05) higher than Madura (2.0%) and was not significantly different (P> 0.05) with PO (3.8%). The percentage of spermatozoa hyper activity fluctuate during cold storage. Bali bulls showed the spermatozoa hyperactivity value tends to increase during cold storage. Nevertheless, the spermatozoa hyperactivity on Madura bulls rises at the beginning of storage and then decreased up to H5 of storage. The percentage of hyperactive spermatozoa on PO bulls relatively stable during cold storage.

The categories of spermatozoa hyperactivity with SCA 5.2. are has VCL > 150 μm / s; LIN <50% and ALH> 7 μm. Results of previous studies have hyperactive spermatozoa standards are slightly different. Shibahara *et al*., (2003) stated standards on human spermatozoa hyperactivity is VCL> 100 μm / s, LIN <60% and ALH>5 μm. Meanwhile, Ripp *et al*., (2003) is a VCL> 100 μm/s, LIN <60% and ALH> 7.5 μm. Sohail *et al*., (2012) suggest that

during the freezing there is impairment of motility, progressive motility and velocity. An increase in the value of BCF and characterized by changing patterns of sperm movement becomes less linear or progressive, then it is an indication of hyper activated movement. Kathiravan *et al*., (2011) stated that there is an indication of spermatozoa hyperactivity when spermatozoa have ALH> 7 μm and VCL> 70 μm /s. In ideal conditions, fresh semen has a hyperactive spermatozoa relatively low but will increase during capacitation. Capacitation event is accompanied by changes in sperm membrane structure followed by an increase in Ca2+influx which trigger hyperactivity and ultimately sperm acrosome reaction (Schmidt and Camp, 2004).

ALH and BCF

Parameters of ALHand BCF describe spermatozoa wave pattern which is determined by averaging the track and groove. The observation parameters of ALH and BCF of liquid semen during cold storage on PO, Bali and Madura bulls with CEP-2 diluent are listed in Table 6.

Different superscripts in the same row indicate significant differences (P <0.05)

In the Table 6 showed that there was no significant difference (P>0.05) of ALH between breeds of bull at H0 and H5 of storage. Meanwhile, BCF value between breeds showed significantly different (P<0.05) at H0. The value of BCF at H0 on Madura bull (13.0 Hz) significantly different (P<0.05) higher than PO (11.1 Hz) and Bali bull (10.6 Hz). Sarastina (2006) which states that the Madura cattle have BCF value is the highest among other types of cattle. BCF value indicates the frequency of movement of spermatozoa.

The ALH value obtained by mathematically calculating the average maximum distance of the groove and the maximum excursion of the track. The increase in ALH is characterized by increased movement of the head to the side that forms the pattern of stars (star-shaped pattern). The ALH parameter indicates the average width of the oscillation (vibration) spermatozoa head while swimming (Kathiravan *et al.*, 2011). Tardif *et al* (1996) stated that the value of ALH sharply increased after spermatozoa cooled. While the BCF is the number of times the path crosses an average sperm per second groove. Kathiravan *et al.*, (2011) stated that the BCF is a useful parameter to identify changes in the pattern of flagellar beat. During cold storage there was no indication of changes in flagellar beat on all bull breeds. The BCF value is influenced by the value of the frame rate (FR) on the CASA and VAP (Kathiravan *et al.*, 2011; Perreault, 2002).

CONCLUSION

Sperm motility of liquid semen at early cold storage showed many different between breeds. Sperm motility of Madura and Bali bull showed similar value (VCl, VSL,VAP) which higher than PO bull. Sperm motilityon PO semen, Bali and Madura bull with CEP-2 diluents at the end of cold storage showed same value (motility, progressive motility, VCL, VSL, VAP, LIN, STR, WOB, ALH). Madura bull showed the best motility with low of hyperactive sperms. The use of CEP-2 diluents in liquid semen may support sperm motility in PO, Bali and Madura beef bull up to the 5th day of cold storage.

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