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Effect of Caffeine on Motility of Epididymis Spermatozoa of Bali Bull in Slaughterhouse Cibinong

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Abstract

Abattoir is the place to get meat but also a source of potential genetic sperm. Sperm from a slaughterhouse has low motility. Sperm motility can be improved by adding caffeine to the thinner before being used for fertilization. Caffeine is an alkaloid compound that can increase energy through a cAMP cycle. The method used is the testis of 12 cows Bali taken from a slaughterhouse Cibinong and do frozen sperm. Frozen sperm is analyzed using a computer-assisted sperm Analyzed (CASA) who had been treated caffeine 0, 2, 4, and 6 mg/ml. Fertility frozen epididymis sperm was tested using in vitro fertilization. Results were analyzed using a completely randomized design (CRD) with four treatments unidirectional pattern three repetitions. The results showed that the treatment with the addition of caffeine to the thinner of the yolk tris egg yolk epididymis sperm, there was no difference in motility, *recovery rate*, Curvilinear velocity (VCL), average path velocity (VAP), and straight-line velocity (VSL).

Keywords: cauda epididymis sperm, motility, caffeine

A. Introduction

Increasing population growth will be followed by an increased need for animal protein (meat). The fulfillment of meat pushed the number of slaughter cattle in particular. The level of cuts is excellent when the preservation of genetic resources does not follow. It will lead to

extinction. In Indonesia, Bali cattle are cattle who preferred because it has excellent performance and carcass were quite high (57%). Preserve the genetic resources would be utilized, then the sperm collected should be possible in doing the storage process. Frozen semen is the correct method and can be used for artificial insemination (AI) with unlimited time. Epididymis sperm is sperm that is still young, so it has low mobility. The storage process can also result in decreased motility. To overcome these problems, sperm, before it is used for fertilization, should be added compounds to improve motility.

Motility is the main factor in fertilization. Sperm can move progressively forward fallopian tube will quickly get to the point of fertilization. Sperm takes long enough to get to the fallopian machine, while epididymis sperm have low motility. In vitro, fertilization is an efficient method for epididymis sperm. However, to improve epididymis sperm after the freezing process, it must be added additive compound that functions as a stimulant. Caffeine is one of the compounds that can be added to the post-thawing frozen sperm.

Caffeine is an alkaloid found in plants formed of white powder with the mechanism of action to inhibit the activity of nucleotide phosphodiesterase, which naturally cAMP levels are relatively low due to the activity of nucleotide phosphodiesterase, so the addition of caffeine can suppress the activity of nucleotide phosphodiesterase that lead cAMP levels to increase (Hasbi et al., 2011). the cAMP is the energy used to move progressive sperm. Increased sperm motility will support the level of fertility of sperm.

B. Methodology

1. Research Design

This study used a completely randomized design (CRD) factorial pattern of 4 x 3 with three replications (semen collection).

2. Research Procedures

a. Semen collection and preparation

Bali cattle testes collected from a slaughterhouse. Testes incorporated into the physiological sodium liquid and transported to the laboratory. Testicular cleaned and cut into sections cauda epididymis. Slice cauda epididymis tris incorporated into the solution for one minute, grab the piece cauda epididymis. Add the egg yolk in a solution of tris and equilibration at a temperature of 5^0C for 2 hours. Pack in straw with 0.25 ml size, close the end of the straw with a hot plate and pressed with tweezers, then straw is placed in a Styrofoam plate in nitrogen vapor for 20 minutes and put in liquid nitrogen (-1960C).

b. Motility examination of spermatozoa

Frozen semen cauda epididymis in thawing in warm water temperature of 37^0C and then centrifuged at a speed of 1800 rpm for 5 minutes. Separate the plasma liquid then add tris solution of 1 ml and caffeine with T0 (0 mg / ml), T2 (2 mg / ml), T4 (4 mg / ml), and T6 (6 mg / ml).. Analysis of sperm motility by dropping a sample in a glass object that has been treated with a micropipette 1µl, then covered with a cover glass, is then observed by computer-assisted sperm motility Analyzed (CASA).

3. Research Parameters

The parameters of this research were motility of spermatozoa (%), curvilinear velocity (VCL); average path velocity (VAP); and straight-line velocity (VSL).

4. The technique of Data Analysis

The differences between concentrations were compared, and results were expressed as mean ± Sd. Analysis of variance (ANOVA) using the SPSS software version 18 with Tukey test was performed to verify statistical significance. The p-values of <0.05 were considered as statistically significant (Trihendradi, 2010).

C. Results and Discussion

Assessment using CASA in this study saw three variables are correlated with fertility that is curvilinear velocity (VCL) shows the velocity of sperm in a minute on the trajectory curve,

average path velocity (VAP) is the velocity of sperm in a minute on the track the average flow and the straight-line velocity (VSL) is the velocity of sperm in a minute straight trajectory (Sarastina et al., 2014). Calculation of post-thawing sperm motility with the CASE method shown in Table 1.

Table 1 Cauda epididymis sperm motility post-thawing

Variable	Caffeine Treatment (mg/ml)			
	T0	T2	T4	T6
Motility(%)	22,73 ± 6,39[a]	27,37 ± 2,14[a]	30,70 ± 0,34[a]	23.04 ± 5,60[a]
Recovery rate(%)	43,11±12,11[a]	51,91 ± 4,07[a]	58,23 ± 0,64[a]	43,70±10,62[a]
VCL (µm/s)	84,96 ± 1,37[a]	85,06 ± 4,68[a]	87,96 ± 11,73[a]	87,59 ± 2,39[a]
VAP (µm/s)	40,88 ± 3,68[a]	46,21 ± 1,72[a]	41,80 ± 2,34[a]	39,15 ± 5,39[a]
VSL (µm/s)	29,20 ± 3,28[a]	33,87 ± 2,63[a]	30,92 ± 1,39[a]	29,20 ± 4,15[a]

Description: The same superscripts in the same row showed no different (P> 0.05). T0 = 0mg / ml, T2 = 2 mg / ml, T4 = 4mg / ml, and T6 = 6mg / ml. Curvilinear velocity (VCL), average path velocity (VAP), and straight line velocity (VSL).

The addition of caffeine at levels of 0, 2, 4, and 6 mg/ml no effect (P> 0.05) to the cauda epididymis sperm motility post-thawing shown in Table 1. Epididymis sperm are immature sperm that still has granular cytoplasmic droplet and lets not resistant to changes in temperature (cold sock) during the freezing process, thereby granting post thawing caffeine does not affect motility. It is supported by a statement Sukmawati (2014), which states that the freezing process may cause a decrease in motility (50-60%) and viability (20-23%). Value motility resulting in Table 1 ranged from 23% to 30%, thus enabling the sperm can still fertilize. It is consistent with the statement Putranti (2016) treatment caffeine level 4 mg/ml have value fertilization 37,50%.

Recovery rate (RR) is the ability to recover sperm after freezing by comparing the percentage of fresh motile sperm with post-thawing (Garner dan Hafez, 2000). The results of further tests with Tukey showed that caffeine treatment in the cauda epididymis sperm was not different (P>0,05) in the RR of all treatments with T0 (43,11±12,11%), T2 (51,91 ± 4,07%) , T4 (58,23 ± 0,64) and T6 (43,70±10,62). These results indicate that the egg yolk tris of sperm in epididymis cauda is very efficient to use because it shows a *Recovery rate* of around 50%.

Value VCL, VAP, and VSL in Table 1 show the results did not differ (P> 0.05) among all treatments caffeine. It was because motile sperm have the same strength as it moved forward, so it has the average no different. VSL grades (29-33 µm/s) and the value of VAP (39-46 µm/s) shown in Table 1 is still within the normal range, so that they can move to fertilize the egg. Normal standards according Royere (1996) in Arifiantini (2012) is VSL> 30µm/s and VAP> 25µm/s.

D. Conclusion

The results showed that the treatment with the addition of caffeine to the thinner of the yolk tris egg yolk epididymis sperm, there was no difference in motility, *recovery rate*, Curvilinear velocity (VCL), average path velocity (VAP), and straight-line velocity (VSL).

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