



The Viability of Bali Cattle Epididymal Sperm with the Addition of Caffeine in Tris-Egg Yolk Diluents

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ARTICLE INFO

e-ISSN: 2548-3803

p-ISSN: 2548-5504

Vol. 5, No. 2, December 2020

URL: <https://dx.doi.org/10.31327/chalaza.v5i2.1362>

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Abstract

The research aimed to examine the effect of caffeine's addition on diluents on Bali cattle epididymis' survival or sperm viability to get the right amount of caffeine. The epididymis collection was taken from the Slaughterhouse of Ternate City. Analysis of sperm vitality or viability was carried out in the Biotechnology Laboratory of Universitas Khairun. The methods in this research were by preparing egg yolk Tris solution treated with caffeine P0 (0 mg/ml), P2 (2 mg/ml), P4 (4 mg/ml), and P6 (6 mg/ml) with four replications, and then the epididymal sperm was inserted into each treatment. The epididymal sperm viability was calculated by evaluating the percentage of the number of living sperm. It compared to the dead sperm, which were observed microscopically by eosin-nigrosin staining. The data obtained were analyzed by one-way analysis of variance (ANOVA) to see if there were significant differences ($P < 0.05$) or very real ($P < 0.01$), the analysis were then continued with the Tukey-W-Procedure test with SPSS 18. The results show that administration of caffeine 2 mg/ml and 4 mg/ml (86.95% and 86.95%) have a significant effect on the sperm viability of epididymis compared with 0 mg/ml and 6 mg/ml (69.38% and 69.91%) treatments.

Keywords: sperm, epididymis, caffeine, viability.

A. Introduction

Bali cattle are native Indonesian cattle that can grow well with feed with low nutritional value and are adaptive to Indonesia's climatic conditions. 90% of Bali cattle maintenance in Indonesia is in smallholder breeders with very limited feed, so productivity is generally low (Baliarti, 1991).

Bali cattle are a breed of cattle that are mostly raised in Eastern Indonesia. Bali cattle are large ruminants which have many advantages, namely that they are easy to adapt in unfavorable environments with minimal feed and low quality, but have the advantage of being able to convert good feed in their bodies so that they show good performance with high carcasses of 50-55% of body weight

Slaughterhouse has the function of producing meat to meet the animal protein needs of the community. The slaughterhouse of Ternate City can slaughter ten cows in a day. Bulls are cattle that are slaughtered, which, of course, should have a good level of health and performance. Apart from produce slaughterhouse meat, bulls also produce germ-plasm, namely spermatozoa.

The testes are the reproductive organs of bulls not included in the carcass, so their benefits are often overlooked. Spermatozoa that have been mature or produced in the testes will be stored in the *cauda epididymis*. However, *cauda epididymis* of spermatozoa had low motility. It impacts the low viability of sperm or the life or death of sperm. The increased motility of *cauda epididymis* spermatozoa will increase the number of living sperm. So spermatozoa diluents need to be added with additives that function as precursors. The addition of diluent in sperm processed until a freeze is expected to provide better results. The addition of the diluent in the process of pickling liquid semen can maintain the quality of sperm during freezing to thawing back (Hastuti & Suparman, 2016)

The use of caffeine in the diluent is expected to stimulate spermatozoa dormant. Specially spermatozoa are in the cauda epididymis after the shelter becomes alive motile (Soeparna & Nurcholidah, 2014). Thus sperm collection is not only from live bulls, but it can also be done in the slaughterhouse for the benefit of artificial insemination (Pamungkas, 2012). Hastuti & Junaedi (2019) said that diluents are important in packing semen in the form of straw or frozen ampoules. It is expected that the semen quality and viability of spermatozoa during the freezing process can be maintained.

B. Methodology

1. Materials of Research

The materials are petri dish, object-glass, cover glass, scalpel, blade, measuring cup, Neubauer counting chamber (Neubauer hemocytometer), micropipette, microtip, tweezers, pH meter, cool box, and microscope, epididymal cauda sperm, 0.9% NaCl, eosin-nigrosin, streptomycin, penicillin, Tris, egg yolk, fructose, formol saline, citrate acid, glycerol, and caffeine.

2. Procedure of Research

First of all, doing the collection of *Cauda epididymis* spermatozoa. The testes were collected from 12 male Bali cattle. The testicles that have been cleaned of the skin are then clamped using arterial forceps in 4 parts, namely the corpus, head, cauda, and tube deference. It was then entered into a physiological 0.9% NaCl solution, which has been given the antibiotic streptomycin 0.1 g/l and penicillin 0.06 g/l and put in a cool box filled with warm water with a temperature of 37°C to be brought to the laboratory. The *cauda epididymis* was separated from the testis. The next step is to slice the cauda epididymis tissue. Then, doing sucks the white liquid on the cauda epididymis using a micropipette. Placed it in an object-glass and analyzed its characteristics. (Putranti, 2016).

3. Parameters of Research

The parameter measured in this study was the viability. Spermatozoa that have good microscopic values with motility of 50% were further processed, namely with the tris-egg yolk diluents and treated with the addition of caffeine P0 (0 mg/ml), P2 (2 mg/ml), P4 (4 mg/ml) and P6 (6 mg/ml). Sperm were dropped into a glass object treated with caffeine with a 10µl micropipette a 2 second to evaluated. Dropped the *cauda epididymis* sperm using a micropipette and eosin-nigrosin drops, then mixed with a ratio of 1: 3, homogenized quickly, made a smear preparation, and viewed using a light microscope. Calculated the percentage of live and dead sperm, with sperm that are red-colored were dead sperm, and white ones were live sperm, counted on 200 sperm cells (Arifiantini, 2012).

4. Data Analysis

This study used a completely randomized design (CRD) with four treatments, namely, (1) decaffeinated control, (2) 2 mg caffeine levels, (3) 4 mg caffeine levels, and (4) 6 mg caffeine

levels. Each treatment was analyzed for living spermatozoa with intact acrosomes. It saw the living spermatozoa with damaged and dead acrosomes. These variables were analyzed every 24 hours in each treatment with three replications. The data obtained were analyzed by one-way analysis of variance (ANOVA) to see if there was a significant difference ($P < 0.05$) or very real ($P < 0.01$), the analysis were then continued the Tukey-W-Procedure test with SPSS 18 (Trihendradi, 2010).

C. Result and Discussion

Viability is a sperm analysis to determine the vitality of sperm using eosin-nigrosin staining (Toelihere, 1993). Live sperm will have a lighter color, while dead sperm will be darker in color. The effect of caffeine on sperm viability can be seen in Table 1.

Table 1. Sperm viability of cauda epididymis with caffeine treatment (mean + SD)

Parameters (%)	Caffeine Treatment			
	P0	P2	P4	P6
H (Whole acrosome)	69.38 (290/418) ^b	87.50 (210/240) ^a	86.95 (200/230) ^a	69.91 (230/329) ^b
H (Broken acrosome)	9.57 (40/418) ^{bc}	8.33 (20/240) ^b	3.48 (8/230) ^a	8.81 (29/329) ^c
Die	21.05 (88/418) ^b	4.17 (10/240) ^a	9.57 (22/230) ^a	21.28 (70/329) ^b

Note: different superscripts in the same row indicate significantly different ($P < 0.05$). T0 = 0mg/ml, T2 = 2mg/ml, T4 = 4mg/ml and T6 = 6mg/ml. H: Alive

The results in table 1 showed that live sperm with full acrosome by Tukey's follow-up test, P2, and P4, are significantly different ($P > 0.05$) compared to T0 (P0) and T6 (P6). It shows that the caffeine treatment of 2 mg/ml and 4 mg/ml can maintain the intracellular ion's stability so that the cells' metabolic balance runs normally. The acrosome that is still attached to the sperm head is very important because a hydrolytic enzyme will shed the zona pellucid in oocytes (Elder & Dale, 2003).

Live sperm without acrosome will have difficulty penetrating the zona pellucid because no enzymes can penetrate the oocyte cell wall. (Gagnon & de Lamirande, 2006). The results of Tukey's follow-up test of P4 were lower ($P < 0.05$) compared to P0, P2, and P6. A caffeine level of 4 mg/ml can increase acrosome hyper-activation according to the energy required to move. In contrast, an increase in caffeine level of 6 mg/ml results in acrosome damage. The increased concentration of Ca^{2+} results in uncontrolled hyper-activation of sperm so that many of the acrosome's outer layers are detached or damaged (Kito & Ohta, 2005).

Dead sperms in the study in Table 1 show that treatments at P0 and P6 are significantly different ($P < 0.05$) compared to P2 and P4. Increasing the caffeine level will increase metabolism in the mitochondria, which will produce energy and lactic acid with toxic properties because the pH will become more acidic. As a result, the sperm will die quickly (Mukminat et al., 2014). Figure 1 below shows living sperm with acrosome and dead sperm with acrosome.

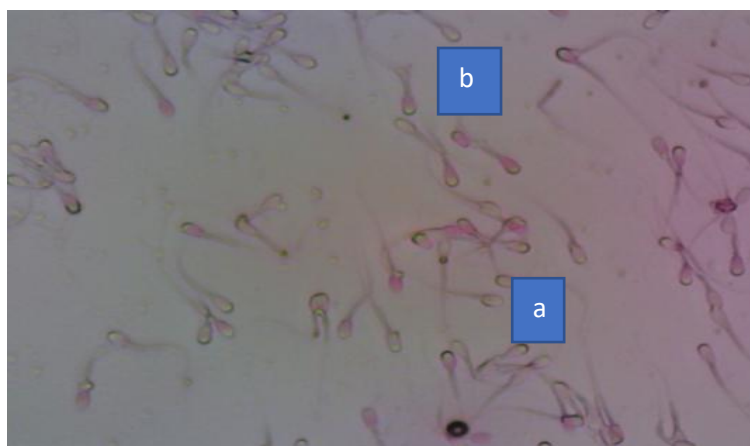


Figure 1. Sperm viability: acrosome life and acrosome death with 400x magnification of eosin-nigrosin staining. a). Living sperm with acrosome, b). Dead sperm with acrosome.

In Figure 1, it can be seen that there are two colors on the head of the sperm, pink and white. These colors indicate the state of the sperm, either dead or alive, in which the dead ones are shown as pink or darker in color, and the live sperms show a lighter color on the head. The acrosome is found at the end of the head with a darker-colored line or black curves in dead sperm and colorless in the remaining living sperms (Soeparna & Nurcholidah, 2014).

The difference in color in the sperm is that living sperm does not absorb the eosin-nigrosin staining, while dead sperm will easily absorb the staining to be darker in color than live sperm. It is because the dead sperm have high membrane permeability (Arifiantini, 2012). Living sperm with acrosome certainly has a high degree of fertility. In the head, an enzyme can lysis the zona pellucid so that the spermatozoa head can enter the nucleus of the egg or nucleus. Sperm without the acrosome means that the enzyme has been lost before it attaches to the zona pellucid, resulting in sperm being unable to fertilize an egg.

D. Conclusion

The results show that caffeine administration 2 mg/ml and 4 mg/ml have a significant effect on the viability (86.95% and 86.95%) of *cauda epididymis* sperm compared to 0 mg/ml and 6 mg/ml (69.38% and 69.91%) treatments.

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