The effect of Andromed® and coconut water + 20% egg yolk as diluent on semen motility of Belgian Blue cattle

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Abstract. The purpose of this study was observed the potential use of coconut water + 20% egg yolk (CW) as an alternative diluent to replace Andromed®, on semen motility of Belgian Blue (BB) cattle. The sample were fresh semen collected from 3 years old BB using artificial vagina. The fresh semen was diluted into Andromed® diluent (P1) and coconut water + 20% egg yolk (P2). The semen motility (%) was observed at 0, 2, 4, 6, 12, 24 and 48 hours after dilution and then compared between group using t-test. The result showed that Andromed® found to give better motility compared to CW in all observation time. The average of semen motility (%) in accordance with observation time in group P1 was 77.67±2.51, 74.67±2.51, 72.33±2.51, 70.67±2.51, 69.33±1.54, 66.33±2.88 and 63.33±2.88%, respectively. Meanwhile, in P2 was 71.67±2.89, 65.00±5.00, 60.00±5.00, 58.33±2.89, 46.67±7.64, 41.67±7.64 and 26.67±7.64 % respectively. In conclusion, coconut water + 20% egg yolk could be replace the function of Andromed as diluent for AI using liquid semen with maximum storage time no more than 24 hours.

1. Introduction
Belgian Blue (BB) was originated from Belgium with advantage for its double muscle [1]. This double muscle traits occurs due to deletion of 11 bases in exon three of myostatin gene [2, 3]. The mutation occurs naturally in myostatin gene which encode MSTN protein involved in muscle development. Myostatin is a protein member of superfamily that transforms growth factors (TGF) -β and plays an important role in muscle growth and meat quality.

Artificial insemination (AI) could be used to improve genetic quality of its offspring and spread the genetic potential of BB [4, 5]. Artificial Insemination is one of technologies aimed to improve the genetic quality of livestock by assisting delivery of semen into the female reproductive. The AI function by utilizing one ejaculation can produce acceptor many females were inseminated with freezing semen or liquid semen [4]. The success of the IB does not only depend on the quality and quantity of cement that is ejaculated, but also depends on the quality of the diluent used [7].

The fertilization ability of spermatozoa must be preserved or preserved for some long after collected to maintain motility and viability, by therefore semen need to be mixed with diluent solution that guarantees the need physical and chemical and stored on certain temperatures and conditions that maintain the life of the spermatozoa during the desired time for later used as needed [8]. Andromed is commercial instant diluent contains egg yolk free concentrated extender medium for freezing of bull semen and semen of other ruminants, also suitable for the preservation of fresh semen [9]. Andromed
diluent can give the best influence on percentage of motility and percentage of life spermatozoa compared to skim milk [10].

Andromed® is an imported product, so that the price was relatively expensive. Therefore, alternative diluent with high sustainability and lower prices are needed. Alternative diluents are expected to be more sustainable than Andromed® diluents, i.e., prices are relatively cheap, easy to obtain, and can maintain the quality of spermatozoa [6]. Coconut water is being used in this research because their high sustainability especially in rural area of Indonesia. Coconut water can be used as diluents because it has simple carbohydrates like: glucose, sucrose and fructose as an energy source of spermatozoa, besides that coconut water is cheaper and easier to get and have complete nutrition [11].

Egg yolks as ingredients extracellular cryoprotectant work as provider of food media, energy sources, and extracellular protective spermatozoa from cold shock because it contains lipoprotein and lecithin [8]. Egg yolks also help to prevent hypermotility and early capacitation of spermatozoa [4,12]. The purpose of this study was to observe the potential use of coconut water + 20% egg yolk as an alternative diluents Andromed® substitute on semen motility of BB.

2. Material and methods

2.1. Material

The material used in this study consisted of fresh semen, tools, and materials. Fresh semen collected from 3 years old BB Cattle using artificial vagina tool. The ingredients used in this research are Andromed® diluent, coconut water, egg yolk, penicillin-G, streptomycin sulphate, alcohol, Hematoxylin-eosin solution, Hayem solution, aquadest, 15°C ice water, litmus pH paper, aluminum foil, 3 fruit semen tubes, 2 flasks, microscopes (Tension, Germany), optilab, pipette haemocytometer (Assistant, Germany), Neubauer rooms counting, drop pipette, counting device, object glass, coverslip and measuring tape.

2.2. Methods

2.2.1. Semen collecting method. Semen collected from 3 years old BB Cattle using artificial vagina tool two times every week and only one ejaculated, at four days intervals. Fresh semen obtained then tested the quality of the semen macroscopically and microscopically. The result of microscopic and macroscopic BB cattle fresh semen was presented in table 1.

| No | Parameter          | Average      |
|----|--------------------|--------------|
|    | **Macroscopic**    |              |
| 1  | Volume (ml)        | 9.63±1.15    |
| 2  | pH                 | 7.13±0.11    |
| 3  | Color              | Cream        |
| 4  | Consistency        | Thick        |
|    | **Microscopic**    |              |
| 1  | Motility (%)       | 81.00±1.00   |
| 2  | Viability (%)      | 84.33±1.52   |
| 3  | Concentration (x million/ml) | 2,108±0.11 |
| 4  | Abnormality (%)    | 14.5±2.69    |

2.2.2. Dilution method. Andromed® diluted by adding aqua bides in a ratio 1:4 for making Andromed diluent before adding with fresh semen, and then fresh semen added with Andromed® diluent as much as the volume needed (P1). Coconut water poured into measuring tube as much as the volume needed. Eggs are prepared and cleaned using alcoholic cotton 70%. All of albumin are thrown away and whole egg yolk wrapped in membrane vitelline is transferred to filter paper to remove the liquid egg white
left over, and then fresh semen added with coconut water + 20% egg yolk (P2). Bearden and Fuquay (1997) stated that the use of 20% egg yolk and 80% buffer thinners gave the best results on semen dilution.

2.2.3. Data collecting method. After diluted with Andromed® and coconut water + 20% egg yolk, liquid semen put into a refrigerator at 5°C for equilibration, and then motility data are observed every 0, 2, 4, 6, 12, 24 and 48 hours after dilution using a microscope and optilab.

2.2.4. Analysis method. The data of motility on both diluents were analyzed using t-test with SPSS V.16 software

3. Results and discussion
The result of comparison of BB cattle fresh semen motility between Andromed diluent and coconut water + 20% egg yolk was presented in the table 2.

Table 2. Comparison of BB cattle fresh semen motility between Andromed® diluents and coconut water + 20% egg yolk

| Diluent     | Equilibration times (hours) |
|-------------|-----------------------------|
|             | 0       | 2       | 4       | 6       | 12      | 24      | 48      |
| Andromed®   | 77.67±2.51 | 74.67±2.51 | 72.33±2.51 | 70.67±1.54 | 69.33±1.54 | 66.33±2.88 | 63.33±2.88 |
| CW          | 71.67±2.89 | 65.00±5.00 | 60.00±5.00 | 58.33±2.89 | 46.67±7.64 | 41.67±7.64 | 26.67±7.64 |

a, b superscript in the same column showed significant different

The results showed that there was a significant difference (P<0.05) in semen motility between the diluent Andromed® with coconut water + 20% egg yolk at the equilibration time after 2 hours. The motility value by the addition of Andromed® was normal and good. The average of semen motility by coconut water + 20% egg yolk after equilibration also classified as good and it has decreased after 24 hours. Both of these diluents are still suitable for use in AI because the values of motility are more than 40%. The proper semen motility for AI was at least 40% [4].

Andromed® is commercial diluent and suitable for the preservation of fresh semen [9,13]. Andromed® contains vegetable lecithin from soybean extract which plays a role in maintaining and protecting the membrane integrity of the lipoprotein of semen [6,9,14]. Motility of spermatozoa is a measure used as the ability of spermatozoa to fertilize an egg cell [15].

Coconut water was isotonic with completed nutrient content and has buffer properties [8]. Coconut water can be used as diluents because it has carbohydrates simple as an energy source of spermatozoa, like: glucose, sucrose and fructose [11]. The addition of egg yolk to coconut water diluents can protect membrane integrity and maintain the integrity of the ultra-structural spermatozoa [6]. 20% egg yolk and 80% buffer thinners gave the best results on semen dilution [16]. Prove that the highest progressive motility of cow semen was after dilution used 20% egg yolk [15].

Semen were classified as normal and fertile if the motility values ranged from 50-80% [17]. The quality of semen was stated to be good if it had more than 50% motility [13]. The problem that often arises was usually damage to the plasma membrane of spermatozoa due to the formation of lipid peroxidation, damage to the plasma membrane caused a decrease in semen motility [8].

The decrease in motility value after 24 hours was suspected because damage to the plasma membrane and acrosome of spermatozoa. Spermatozoa have good membrane integrity and metabolism, if plasma membrane is damaged, can cause membrane integrity to decrease [4, 6]. As a result, the control of the transport system was interrupted and resulting in decreased metabolism, motility and survival of spermatozoa [16]. Based on this, the use of coconut water + 20% egg yolk as liquid semen for AI should not exceed one day.
4. Conclusion
In conclusion, coconut water + 20% egg yolk could replace the function of Andromed as a diluent for AI using liquid semen with a maximum use of no more than 24 hours.

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