Effects of various diluents on the quality and shelf life of Donggala bull semen

Mirajuddin, Y Duma, M I Mumu, M R Ladjama, Nur A’fia, A M Abas and A Ringgiallo
Department of Animal Science, Faculty of Animal Husbandry and Fisheries, Tadulako University, Indonesia

Corresponding author: mirajuddinm@ymail.com

Abstract. This study aimed to determine the effects of different semen diluent on the quality and storage time of liquid semen of Donggala bull. Semen was obtained from four selected bulls which collected using artificial vagina. The semen diluent is based on Tris aminomethane-citric acid with different concentration of glucose namely P1=0.00g, P2=0.25g, P3=0.50g, and P4=0.75g. Another group of treatment also prepared based on Tris aminomethane-glucose with different concentration of egg yolk namely P5=0%, P6=15%, P7=20%, and P8=25%. The data obtained were then analyzed descriptively. Results showed tris aminomethane-citric acid-glucose diluents had sperm progressive motile at >40% until the day 4 of storage, and tris aminomethane-glucose-egg yolk only able to support sperm life for 2 days, and in P8 group shows 41.02% motile on the day 3. In this study, we found the pattern of the increasing of shelf life affect to the decrease rate of sperm viability and normality.

1. Introduction
Donggala cattle is one of Indonesian native cattle that has contribution to the provision of meat. These cattle have been designated since 2014 as genetic resource for local farms community which spread across the Central Sulawesi Indonesia. Various studies from many aspects are needed to support the efforts to improve the population in both quality and quantity. One of effort is to distribute genetic from superior bull to the community by using frozen of liquid semen for Artificial Insemination (AI) purpose.

The development of diluent formulas provides a way to longer shelf life of semen. Preparation of semen diluent allows exploring the potentials of the bull's reproduction, at almost no risk of venereal disease [1]. The diluent formula plays an important role in the efficient use of superior bulls by increasing the number of AI dose per ejaculate. Moreover, diluent also act to protect and maintain semen quality during storage [2-4], as well as preserving sperm ability to fertilize [5]. Various diluent formulas have been developed in several types of livestock with varying shelf life, for example skimmed milk and tris-citrate were applied in sheep [6], fish-oil-skimmed milk-egg yolk in Kalang buffaloes [7], cauda epididymal plasma (CEP2)- egg yolk in Limousin cattle [8], CEP2- egg yolk in FH cattle [9] and citrate -egg yolk-raffinose in Ongole cattle [2]. CEP2-egg yolk, young coconut water-egg yolk in Madura cattle [4], and tris- egg yolk- isotonic solution commercial in Simental cattle [10].

The use of commercial ready-to-use diluents such as AndroMed® and Bioxcell® requires a high cost. For that reason, it is necessary to find cost alternatives that are cheap and easy to obtain. Some basic media used in the preparation of liquid and frozen semen of ruminants are tris, citrate, egg yolk, glucose,
and skim milk with other additives, and they are prepared in various composition to produce a longer shelf life with quality that is still suitable for AI i.e., motility >40% [11], viability >50% [12] and abnormality <20% [13].

2. Materials and methods

The study was conducted at the Central Sulawesi Livestock Breeding UPTD laboratory and the Faculty of Animal Husbandry, Tadulako University. Four Donggala bulls with an average weight of 380.75±11.32 kg and 3–4-year-old, were used as source of fresh semen. These bulls were selected from community livestock in Donggala and Sigi Regency. They were placed in individual stall (1.5 x 2 m), combination of forage consist of elephant grass and corn stalks (±30 kg/head/day) and concentrates (±5 kg/head/day) were given as feed, and water is always available.

2.1. Semen storage and evaluation

Fresh semen of Donggala bull was collected using artificial vagina and other bulls as libido angler (teaser), and this process was carried out 2-3 times to have enough. The fresh semen obtained then evaluated macroscopically, by observing volume, color, consistency, and pH. Microscopically the semen also evaluated in its concentration, abnormalities, viability, and mass and individual motility. The criteria of semen which processed further, dilution for liquid semen, is had >2+ and >70% for mass and individual motility, respectively [8,11]. The evaluation results of its quality are as follows: cloudy white color, medium-thick consistency, volume 5.11±1.24ml, pH 6.66±0.25, concentration 356.67±255.39 (10⁶/ml), mass motility >2+, individual motility 77.79±0.10%, and live spermatozoa 86.83±15.84%, which made it suitable to be processed/diluted.

2.2. Semen dilution

The semen diluent formula in this study is consist of tris amino methane, citrate, glucose, and chicken egg yolk aged <3 days [4]. Moreover, the diluent formula tested in this study were as follows:

P1 = Tris aminomethane 3.634 g + citric acid 1.99 g + 0 D-glucose
P2 = Tris aminomethane 3.634 g + citric acid 1.99 g + 0.25 g D-glucose
P3 = Tris aminomethane 3.634 g + citric acid 1.99 g + 0.50 g D-glucose
P4 = Tris aminomethane 3.634 g + citric acid 1.99 g + 0.75 g D-glucose
P5 = Tris aminomethane 3.634 g + 0.50 g D-glucose + 0% Yolk
P6 = Tris aminomethane 3.634 g + 0.50 g D-glucose + 15% Yolk
P7 = Tris aminomethane 3.634 g + 0.50 g D-glucose + 20% Yolk
P8 = Tris aminomethane 3.634 g + 0.50 g D-glucose + 25% Yolk

2.3. Observed variables

The fresh semen was diluted according to the tested diluent formula and stored as liquid at ± 5°C. Furthermore, the quality of the liquid semen was observed for seven days (evaluated in every 24 hours). The observed variables are:

a. Progressive motility, the number of spermatozoa that move forward actively, calculated using formula below:

\[
\text{Progressive motile} \, \% = \frac{\text{The number of Spermatozoa moving forward}}{\text{The number of spermatozoa observed}} \times 100
\]

b. Viability is the percentage of live spermatozoa and calculated as follows

\[
\text{Spermatozoa live} \, \% = \frac{\text{The number of spermatozoa live}}{\text{The number of spermatozoa observed}} \times 100
\]

c. Abnormality is the percentage of abnormal spermatozoa, calculated as

\[
\text{Abnormality} \, \% = \frac{\text{The number of abnormal spermatozoa}}{\text{The number of spermatozoa observed}} \times 100
\]
2.4. Data analysis
Data obtained from each diluent formula were then tabulated and analyzed descriptively.

3. Results and discussion

3.1. Spermatozoa motility
The percentage of progressively motile spermatozoa on tested diluent formulas was observed every 24 hours until the day-7 of storage. The results showed a decrease in the rate of motility in all the diluent formulas with increasing shelf life (Table 1). Although this phenomenon was slower in tris-citrate-glucose (P1, P2, P3, and P4) in comparison to tris-glucose-egg yolk diluents (P5, P6, P7, and P8). The P1-P4 diluents maintained the quality of liquid semen with > 40% progressive motility on the shelf life for 4 days, at 50.75%, 43.38%, 54.68%, and 52.42% respectively, where P1-P3 were close to 40% on the day-5. Meanwhile, diluents P5, P6, and P7 only maintained >40% progressive motility for 2 days and P8 was up to the day-3 at 64.08%, 54.54%, 47.82%, and 41.02%, respectively. Therefore, tris-citrate-glucose is a better semen diluent. Especially in P1, P2, and P3 the combination of tris-aminomethane-citric acid is more effective as buffer in maintaining or slowing down the decrease in its pH. This phenomenon maintains the metabolic activity of spermatozoa and makes the decrease in motility become slower and vice versa. This is in line with previous studies [1, 15] which reported that during preservation and storage of semen, the metabolic activity of spermatozoa tends to increase, triggering the production of lactic acid and other acidic substances, therefore the extracellular environment becomes more acidic causing a decrease in pH.

Table 1. Progressive motility percentage of Donggala bull spermatozoa in various diluents and shelf life

| Diluent | 1     | 2     | 3     | 4     | 5     | 6     | 7     |
|---------|-------|-------|-------|-------|-------|-------|-------|
| P1      | 75.99 | 69.93 | 67.75 | 50.75 | 37.26 | 30.17 | 22.48 |
| P2      | 70.92 | 64.96 | 59.43 | 43.38 | 39.68 | 28.07 | 12.06 |
| P3      | 64.77 | 64.21 | 61.61 | 54.68 | 38.95 | 33.56 | 21.02 |
| P4      | 64.54 | 56.50 | 55.69 | 52.42 | 23.73 | 22.69 | 0.00  |
| P5      | 70.00 | 64.08 | 14.75 | 0.00  | 0.00  | 0.00  | 0.00  |
| P6      | 61.23 | 54.54 | 32.65 | 21.28 | 14.70 | 0.00  | 0.00  |
| P7      | 53.89 | 47.82 | 37.50 | 29.18 | 22.07 | 17.15 | 0.00  |
| P8      | 54.22 | 46.67 | 41.02 | 32.91 | 27.50 | 20.93 | 17.75 |
| Average | 64.45±7.9 | 58.59±8.5 | 46.30±17.9 | 35.58±18.7 | 25.49±13.7 | 19.07±12.9 | 9.16±10.3 |

The motility of spermatozoa obtained in this study was higher than the results in the earlier study [10] which obtained >40% only for 2 days of shelf life while using egg yolk tris + 40% CIS (commercial isotonic solution) diluent in Simental cattle. However, the results were lower than on Madura cattle [4], which utilized cauda epididymal plasma 3 (CEP-3) + 20% egg yolk diluent and maintained >40% spermatozoa motility until day 8 at 40.50±6.43%. Also, diluents using young green coconut water + 20% egg yolk and young green coconut water + 20% egg yolk + 0.4% egg white + 2% fructose lasted until the day-6 at 40.50±10.12% and 40.00±8.50% respectively. Young green coconut water + 20% egg yolk + 0.4% egg white + 1% fructose persisted till day 5 at 48.50±7.84%.

3.2. Spermatozoa viability
The viability or percentage of live spermatozoa in various diluent formulas was also observed at every 24 hours until the day-7 of storage (Table 2). The viability decreased differently with increasing shelf life in all the formulas. As with individual motility, the decrease in tris-aminomethane-citric acid-glucose (P1, P2, P3, and P4) was slower than in tris-aminomethane-glucose-yolk (P5, P6, P7, and P8) diluents.
According to the study before [12], the percentage of live spermatozoa that are good for AI is more than 50%. Based on that, diluents P1, P2, and P3 maintained spermatozoa viability >50% for 7 days at 66.67%, 52.66%, 65.86%, and P4 at 57.45% for a 6-day shelf life. Meanwhile, the tris aminomethane-glucose-yolk diluents, P5, P6, and P7, only had 2 days at 20.73% abnormalities, P6 and P7 was only 1 day at 19.56% and 16.98%, respectively, and P8 lasted for 3 days at 19.8%. This means that the tris aminomethane-citric acid-glucose diluent is better in maintaining spermatoza viability in semen. The conditions to become more acidic as the shelf life increases. This situation is known to be toxic to the health of spermatozoa, which sometimes lead to its death [14]. This is controlled by the presence of a better buffer (tris aminomethane-citric acid), which slows down the decrease in spermatozoa viability. Therefore, tris aminomethane-yolk diluent is more effective in protecting spermatozoa in the semen cryopreservation/freezing process [15].

3.3. Spermatozoa abnormality

Percentage of abnormal spermatozoa in several diluent formulas was observed every day until the day 7 (Table 3). The spermatozoa abnormality as observed in Table 3 increased variably with increasing shelf life in all diluent formulas.

**Table 2. Spermatozoa viability percentage of Donggala bull in various diluents and shelf life**

| Diluent | Shelf life (day) |
|---------|-----------------|
|         | 1               | 2               | 3               | 4               | 5               | 6               | 7               |
| P1      | 93.23           | 90.48           | 90.12           | 81.58           | 75.39           | 70.84           | 66.67           |
| P2      | 93.18           | 88.50           | 85.89           | 79.78           | 75.33           | 72.50           | 52.66           |
| P3      | 94.89           | 90.87           | 87.13           | 87.21           | 86.91           | 86.67           | 65.86           |
| P4      | 88.74           | 87.68           | 83.79           | 80.71           | 63.73           | 57.45           | 0.00            |
| P5      | 77.24           | 67.65           | 29.48           | 0.00            | 0.00            | 0.00            | 0.00            |
| P6      | 70.05           | 60.17           | 47.61           | 45.31           | 40.32           | 0.00            | 0.00            |
| P7      | 68.07           | 65.68           | 36.37           | 34.60           | 34.57           | 30.55           | 0.00            |
| P8      | 75.75           | 72.30           | 61.76           | 55.55           | 44.20           | 43.20           | 30.65           |
| Average | 82.64±11.1      | 77.88±12.7      | 65.27±24.8      | 58.09±30.4      | 52.56±28.4      | 45.15±32.9      | 26.98±30.9      |

The rate of increase in spermatozoa abnormality was slower in the tris aminomethane-citric acid-glucose diluents (P1, P2, P3, and P4) than in the tris aminomethane-glucose-yolk formula (P5, P6, P7, and P8). Diluents P1 and P2 slowed down spermatozoa damage <20% up to 6 days at 18.75 and 16.36%, respectively. Meanwhile P3 was 14.28% at 7 days of shelf life and P4 15.62% at day 5. The P5 was less than 1 day at 20.73% abnormalities, P6 and P7 was only 1 day at 19.56% and 16.98%, respectively, however, P8 lasted for 3 days at 19.8%. This means that the tris aminomethane-citric acid-glucose diluent is better at preventing spermatozoa damage in Donggala bull.

**Table 3. Percentage of spermatozoa abnormalities in Donggala bull in various diluents and shelf life**

| Diluent | Shelf life (day) |
|---------|-----------------|
|         | 1               | 2               | 3               | 4               | 5               | 6               | 7               |
| P1      | 8.33            | 11.87           | 15.79           | 17.07           | 18.51           | 18.75           | 27.85           |
| P2      | 6.42            | 7.50            | 10.61           | 14.60           | 15.91           | 16.36           | 31.68           |
| P3      | 7.32            | 8.23            | 10.00           | 10.46           | 11.22           | 13.75           | 14.28           |
| P4      | 10.64           | 10.82           | 10.95           | 15.49           | 15.62           | 21.05           | 0.00            |
| P5      | 20.73           | 24.70           | 33.34           | 0.00            | 0.00            | 0.00            | 0.00            |
| P6      | 19.56           | 24.71           | 24.71           | 24.71           | 34.38           | 0.00            | 0.00            |
| P7      | 16.98           | 21.56           | 26.59           | 26.59           | 26.59           | 27.78           | 0.00            |
| P8      | 15.34           | 19.78           | 19.80           | 20.80           | 23.11           | 30.15           | 30.16           |
| Average | 13.17±5.7       | 16.15±7.3       | 18.97±8.6       | 16.22±8.5       | 18.17±10.3      | 15.98±11.3      | 13.00±14.8      |
4. Conclusion
The diluent formula based on tris aminomethane-citric acid-glucose is better in maintaining liquid semen quality of Donggala bull than tris aminomethane-glucose-yolk. The results showed that tris aminomethane-citric acid-glucose maintained motility >40% till the day 4, viability >50% with 7 days shelf life, abnormality <20% for 6-7 days. Meanwhile, Tris aminomethane-glucose-yolk was only able to maintain motility for 2-3 days, viability >50% for 2-4 days, and abnormality <20% only for 1-3 days shelf life.

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