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**Abstract**. The objective of this study was to compare chicken egg yolk and duck egg yolk in tris-citric acid extender on the quality of post-thawing Bali bull semen. This study was using Bali bull for collecting the semen. The semen was collected five times using an artificial vagina. Collected semen was then divided into two groups at each collection. The group 1 (P1), the semen was extended using chicken egg yolk in tris-citric acid and group 2 (P2) was extended using duck egg yolk in tris-citric acid. The parameters measured in this study were sperm motility, viability, and abnormality. The data obtained were tested by using the paired-T test with five replications. The results of this study showed that there was no significant difference ($P > 0.05$) between the two treated groups for motility and abnormality, but the viability differed significantly ($P < 0.05$) among the two groups after thawing. The mean percentage of sperms motility and abnormality after thawing in P1 and P2 extenders did not differ significantly (60.0 vs. 57.0% and 12.0 vs. 13.0%). However, the viability of the sperms had significantly ($P < 0.05$) higher in P1 than in P2 (74.0% vs. 70.0%). In conclusion, the use of chicken egg yolk and duck egg yolk in tris-citric acid extender could maintain the quality of Bali bull sperms.

## 1. Introduction

Artificial insemination (AI) techniques have spread and extend to lower middle-scale farms, including the development of Bali cattle program in South Sulawesi Province in Indonesia. However, the availability of Bali bull frozen semen in this region is still limited and as a result, the use of this semen has not been widely yet as massive as the other frozen semen, in which the other is easier to obtain from the other region. The reason for this limitation is due to low production from the AI station. Therefore, in the future, it is necessary to produce Bali cattle frozen semen locally in order to develop Bali cattle in the region. To produce frozen Bali bull semen locally, it is necessary to use extender in order to increase the volume of semen and increase the number of straws produced.

In this region, the extender used for extending the semen is mainly imported and it rarely makes its own extender. Therefore, it is necessary to produce extender that can compete with imported extender

by themself and avoid importing expensive extender. One of the local extender usually used is egg yolk in citric acid. This extender is still necessary to increase the quality to become an extender that having a high quality in which be able to maintain the quality of semen.

Usually, the quality of liquid semen has decreased quickly, especially during processing and collection in the field. Therefore, the role of the raw material for the cryo-protectant extender is needed to maintain sperm motility, viability, and abnormalities. Currently, researchers often use the components in making cryo-protectant extenders are glycerol [1] and egg yolk [2,3].

Several glycerol types, such as ethylene glycerol, propylene glycerol, and dimethyl sulfoxide, have a function to prevent cell membrane penetration and cell toxicity [4]. Also, egg yolk contents such as phospholipids, cholesterol, and low-density lipoprotein, protect spermatozoa from exposure to cold shock during the freeze-thaw process. Therefore, this study aimed to test the comparison of using egg yolk duck and commercial chicken egg yolk in the citric acid on the motility, viability, and abnormalities of post-thawing Bali bull semen.

## 2. Materials and methods

### *2.1. Materials*

The main material used in this study was Bali bull for collecting the semen. Preparation of extender and preservative media using sterile aquadest, KY-Jelly, commercial chicken egg yolk, duck egg yolk, tris aminomethane, citric acid, fructose, streptomycin, penicillin, 0.9% NaCl, and glycerol. The types of dyes used were eosin and negrosin. Semen was packaged using a mini straw with a capacity of 0.25 mL.

### *2.2. Methods*

*2.2.1. Dilution preparation.* The diluent used in this study consisted of two main types of extenders; egg yolk of commercial chickens in tris-citric acid (P1) and egg yolk of duck eggs in tris-citric acid (P2). Preparation of egg yolk in tris-citric acid extender begins by weighing 3,634 Tris, 0.50 g glucose and 1.99 g citric acid, then dissolving it in 100 mL aquadest, then heating it to 37°C. The diluent is divided into two separate containers containing 50 mL each. In the first container, 20 mL of commercial chicken egg yolk was mixed, and the second container is mixed with 20 mL of duck egg yolk. The diluent was homogenized using a steerer for 30 minutes. In the last step, 0.1 g of penicillin and streptomycin in each container were added, then homogenized for 15 minutes. The diluent was kept at room temperature or 37°C and ready to use.

*2.2.2. Semen collection.* Semen was collected twice a week in the morning using an artificial vagina. Immediately after collection, semen was evaluated macroscopically and microscopically. That semen met the criteria both macroscopically and microscopically were subjected to the next process.

*2.2.3. Semen evaluation.* The macroscopic evaluation included volume, color, viscosity, and pH using special indicator paper, then the microscopical evaluation, including mass movement, and was conducted under a microscope with 200× magnifications. The percentage of progressive motility was conducted using computer-assisted sperm analysis (CASA) instrument Androvision® (Minitub-Germany). Sperm concentration was measured using a photometer (SDM 6; Minitub-Germany), whereas their sperm viability and morphology percentages were determined using eosin negrosin staining.

*2.2.4. Semen dilution and packaging.* Dilution of semen was carried out by mixing semen into a certain extender according to the treatments that were added with glycerol. The dilution of semen used the formula for calculating semen to the straw volume of 0.25 mL.

$$\text{Dilution volume} = \frac{\text{Motility} \times \text{sperm concentration} \times 0.25 \times \text{semen volume}}{25 \times 10^6} + \text{Semen volume}$$

*2.3. Parameters*

Parameters for determining spermatozoa quality were based on macroscopic and microscopic evaluation on different semen; fresh, diluted, balanced, and after thawing semen. Thawing was carried out in warm water (37°C) for 30 seconds after 24 hours of storage.

*2.4. Data analysis*

The data obtained tabulated in an excel program such as mass motility, individual motility, and percentage of viability sperm. The two different treated groups were compared using a comparative t-test with an independent sample design [5].

## 3. Results and discussion

*3.1. The quality of Bali bull fresh semen*

In this study, freshly collected semen was immediately measured for quality to determine whether the semen was feasible or not followed up in preservation and freezing. The results of the observations are presented in table 1.

**Table 1**. Macroscopic and microscopic evaluation of Bali bull fresh semen.

| | Parameter | Average (± SD) |
|---|---|---|
| A | Macroscopic | |
| | Volume (mL) | 5.16±0.59 |
| | Acidity (pH) | 6.00±0.00 |
| | Smell | Particular |
| | Color | cream-colored |
| | Viscosity | Medium watery |
| B | Microscopic | |
| | Mass Motility (%) | ++ |
| | Individual Motility (%) | 60.00±0.02 |
| | Concentration (million cells/mL) | 1,398.4±350.83 |

Shortly after collecting, ejaculated semen of Bali bull was evaluated macroscopically in the laboratory. The results show that the macroscopic evaluation of the ejaculated semen used was classified as normal. The average volume was 5.16±0.59 mL, pH, and characteristic odor, with medium-dilute viscosity. To strengthen the macroscopic assessment, the semen quality test was then carried out microscopically. Overall, normal results were obtained based on the mass value of motility (++), 60% motility, and the concentration reached 1,398.4±350.83 cells/mL.

*3.2. The quality of Bali bull sperms at different treated groups*

The motility, viability, and abnormality of Bali bull sperm that were evaluated microscopically at different semen extender; chicken egg yolk (P1) and duck egg yolk (P2) in tris-citric acid are presented in table 2.

*3.2.1. Viability of the sperms.* In table 2 shows that the motility of spermatozoa in the freezing stage had decreased gradually until the pre-freezing stage. A drastic increase in motility occurs during the post-thawing condition. Motility percentage values of the two treatments showed that chicken egg yolk tended to show better values than the duck-egg-yolk as extender. However, the values were not significantly different from the two extenders ($P > 0.05$).

In contrast to the previous study [6] showed that duck egg yolk was able to maintain motility and prolong post-thawing motility in stallion spermatozoa's life span. The egg yolk of duck contains higher protein, lipids and cholesterol than the egg yolk of the chicken. The important role of yolk as an extender in the cryopreservation process is to help protect cells from cold shock, storage factors and help maintain survival. In the future, [6] suggests that further research should be carried out related to the use of yolk from different types of poultry because the effect of feed from poultry affects the quality of the yolk produced.

The decreased spermatozoa motility from fresh semen to post-thawing motility was due to the plasma membrane condition damaged during the freezing phase. A decreased plasma membrane condition occurs during the preservation process, where there was a centrifugation stage. The semen handling process could affect the condition of the plasma membrane [7]. Also, the addition of the yolk in the extender was one factor that reduced the speed of movement of the post-thawing motility of spermatozoa. The decreased in post-thawing motility of spermatozoa ranged from 24–64% [8]. A drastic change in temperature during the freezing phase causes cold-shock resulting the spermatozoa cells cannot adapt to the temperature.

**Table 2.** Motility, viability, and abnormality of Bali bull sperms at different treated groups.

| Parameter | Treatment group | |
|---|---|---|
| | P1 | P2 |
| Motility (%) | 60.0 ± 0.02 | 57.0 ± 19.36 |
| Viability (%) | 74.0 ± 4.95[a] | 70.0 ± 4.83[b] |
| Abnormality (%) | 12.0 ± 1.61 | 13.0 ± 1.14 |

[ab]Superscript at the same row indicates a significant difference ($P<0.05$). P1 = Chicken egg yolk in tris-citric acid; P2 = Duck egg yolk in tris-citric acid

*3.2.2. Viability of the sperms.* Spermatozoa viability provided information on the number of live spermatozoa and is associated with the potential for fertilization. The results showed that the viability at P1 was significantly ($P<0.05$) higher than in P2 with the presentation values of 74 ± 4.95 and 70 ± 4.83, respectively. In general, the two extenders provided a good ability to maintain spermatozoa viability above 60%. This ability was supported by the phospholipid content in the yolk, which protected the plasma membrane and played a role in replacing some of the membrane phospholipids damaged during temperature changes [9].

Testing spermatozoa's viability was necessary because it related to DNA fragmentation and male fertility status. The standard of DNA damage to achieve 'high' fertility status is <15% [10]. In post-thawing motility, cauda epididymal spermatozoa, fragmented DNA status had increased from 3 cells to 15 spermatozoa cells on the observation of 100 spermatozoa cells [11]. This indicated that the potential for DNA damage to the post-thawing motility of spermatozoa is small, but prevention efforts were still needed by adding content in the extender.

*3.2.3. Abnormality of the sperms.* The data in table 2 shows that the average percentage of abnormalities in P1 was lower than that in P2 but did not differ significantly. These results were still considered suitable for further processing because the abnormality presentation was not more than 20%. The standard of spermatozoa abnormality is 20% and not more, so it is suitable for artificial insemination.

The incidence of abnormalities in both the head, mid-piece, and tail abnormalities was completely unaffected by several types of extenders made from tris, citric, and egg yolk [12]. However, the extender was proven to be able to suppress abnormalities in spermatozoa in Buffalo.

In this study, spermatozoa morphology was found with a broken tail, so that it was classified into a type of tertiary abnormality in which this abnormality occurs after ejaculation, treatment during collection, and contamination of hazardous substances [13]. Abnormalities in the tail were closely

related to spermatozoa's immotile, causing the inability to move progressively so that the initiation of natural fertilization was less able to occur [1].

## 4. Conclusion

It can be concluded that the extender made from chicken eggs has a better ability to maintain viability and abnormality than duck eggs, while the motility values of the two diluents have the same results. Based on this study, duck egg yolk and chicken yolk eggs are recommended to be used as materials for making frozen semen extenders. However, further are needed to test the use of yolk from different poultry types due to the quality of yolk content.

## References

[1] Kawakami E, Ozawa T, Hirano T, Hori T and Tsutsui T 2005 Formation of detached tail and coiled tail of sperm in a Beagle dog *J. Vet. Med. Sci.* **67** 83–5
[2] Susilawati T 2011 *Spermatologi* (Malang: UB Press)
[3] Foulkes J A 1977 The separation of lipoproteins from egg yolk and their effect on the motility and integrity of bovine spermatozoa *J. Reprod. Fert*. **49** 277–84
[4] Amann RP 1999 Cryopreservation of Sperm *Encyclopaedia of Reproduction* vol 1st, Ed E Knobile and J D Neill (USA: Academic press)
[5] Steel R G D and Torrie J 1980 *Principles and Procedures of Statistics, Biometrial approach* (Singapore: International Book Company)
[6] Clulow J, Maxwell W, Evans G and Morris L 2007 A comparison of duck and chicken egg yolk for cryopreservation of stallion sperm *Aust. Vet. J*. **85** 232–35
[7] Munazaroh A M 2013 *Uji Kualitas Spermatozoa Kambing Boer Hasil Pembekuan dengan Menggunakan Alat Mr. Frosty pada Tingkat Pengenceran Andromed yang Berbeda* [Thesis] (Malang: Universitas Brawijaya)
[8] Ozkavukcu S, Erdemli E, Isik A, Oztuna D and Karahuseyinoglu S 2008 Effects of cryopreservation on sperm parameters and ultrastructural morphology of human spermatozoa *J. Assist. Reprod. Genet*. **25** 403–11
[9] Graham J K and Foote R H 1987 Effect of several lipids, fatty acyl chain length, and degree of unsaturation on the motility of bull sperm after cold shock and freezing *Cryobiology* **24** 42–52
[10] Evenson D P, Jost L K, Marshall D, Zinaman M J, Clegg E and Purvis K 1999 Utility of the sperm chromatin structure assay as a diagnostic and prognostic tool in the human fertility clinic *Hum Reprod.* **14** 1039–49
[11] Masir U, Setiadi M A and Karja N W K 2017 Status DNA dan karakteristik spermatozoa kauda epididimis domba pascapenyimpanan pada suhu 4°C (DNA status and characteristic of sperm cauda epididymal ram after storage at 4°C) *J. Vet.* **18** 167–74
[12] Akhter S, Ansari M S, Andrabi S M H, Ullah N, and Qayyum M 2008 Effect of antibiotics in extender on bacterial and spermatozoal quality of cooled buffalo (*Bubalus bubalis*) bull semen *Reprod. Domest. Anim* **48** 272–8
[13] Menon A G, Barkema H W, Wilde R, Kastelic J P, Thundathil J C 2011 Associations between sperm abnormalities, breed, age, and scrotal circumference in beef bulls *Can. J. Comp. Med.* **75** 241–7