Original Research

Journal of Advanced Veterinary Research
(2026) Volume 16, Issue 1, 106-108

Cryopreservation of Donggala bull semen in Andromed and CEP-2 diluents with the addition of egg yolk in different levels

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ARTICLE INFO

Received: 04 October 2025

Accepted: 21 December 2025

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Keywords:

CEP, Donggala bull, Thawing, Sperm quality, Andromed.

ABSTRACT

This research aimed to know at the quality of Donggala bull semen after thawing, as an effort to preserve local genetic resources in central Sulawesi. This research used five Donggala bulls who had been selected according to their performances. This research employed two kinds of basal diluents Andromed an CEP-2), with five different treatments for each. The diluent formula treatments were as follows: A1= Andromed without egg yolk, A2= Andromed 95% + egg yolk 5%, A3= Andromed 90% + egg yolk 10%, A4= Andromed 85% + egg yolk 15%, A5= Andromed 80% + egg yolk 20%, C1= CEP-2 without egg yolk; C2= CEP-2 95% + egg yolk 5%; C3= CEP-2 90% + egg yolk 10% and C4= CEP-2 85% + egg yolk 15% and C5= CEP-2 80% + egg yolk 20%. The variables observed were motility, viability and abnormality of spermatozoa after thawing. Based on the research results, it showed that the quality of fresh semen from Donggala bull has a volume (6 - !0 ml±1.2), colour (milky white to yellowish white), concentration ($927x10^6$ to $1410x10^6$), with motility (70 to 85 %). Meanwhile, the quality of semen after thawing with the best motility was an average of 58.68±4.46 using Andromed diluent + 15% egg yolk. For C3 diluent, the highest motility (44.2%) was in CEP + egg yolk 10% diluent. The conclusion of this research is that Andromed diluent could maintain the quality of Donggala bull semen after thawing.

Introduction

Local livestock genetic resources (LLGR) are national assets that have a very important and strategic role in agricultural development to support national food security and independence, especially local cattle breeds. Local cattle breeds have the advantage of high environmental adaptation to develop better in their respective distribution and development areas. Donggala cattle as a local cattle breed in the distribution area of Central Sulawesi have excellent environmental adaptability leading to be developed into a superior local cattle herd. Local livestock genetic resources (LLGR), particularly local cattle breeds, are national assets that play a crucial and strategic role in agricultural development to support national food security and independence. Local cattle breeds benefit from a high degree of environmental adaptation, which helps them thrive in their specific areas of distribution and development. Due to their exceptional environmental adaptability (Srivastava *et al*., 2019). Donggala cattle, a native breed in the Central Sulawesi distribution area, have grown to become a superior local cattle herd (Rusdin *et al*., 2024). The plan to move the country's capital to East Kalimantan places Central Sulawesi as the closest region, very ideal and strategic as a buffer for the food supply needs of the new capital. Therefore, Central Sulawesi is improving in such a way as to increase its food production capacity, especially beef cattle.

In this way, Central Sulawesi can become a buffer area for the food needs of the national capital in the future, especially the need for beef cattle quality. In this context, the potential resources of Central Sulawesi, in the form of local cattle, namely Donggala cattle, can be developed as optimally as possible. Central Sulawesi is the closest area and is strategically positioned to serve as a buffer for the new capital's food supply needs in the event that the country's capital is moved to East Kalimantan. Thus, Central Sulawesi is genuinely developing in a way that will boost its ability to produce food, particularly beef cattle. Central Sulawesi can thus serve as a buffer region for the future food requirements of the nation's capital, particularly the demand for high-quality beef cattle. This allows for the best possible development of Central Sulawesi's potential resources, which include local cattle, specifically Donggala cattle.

The existing condition of Donggala cattle in Central Sulawesi currently has at least three issues/problems, namely: a) wide phenotypic variation, characterized by the diversity of Donggala cattle in the community both in terms of skin and hair color, head shape and hump height; b) genetic quality tends to decrease (genetic erosion) as a result of the tendency of negative selection practices by the community by selling large (good quality) Donggala cattle which are ultimately slaughtered and genetic contamination occurred due to mixed breeding where natural breeding with breeds of PO cattle or Bali cattle and artificial insemination (AI) with semen from other breeds of cattle; and c) low production and reproductive performance. Thus, developing and increasing Donggala cattle production on a smallholder scale can be done through improving genetic quality and reproductive performance.

Efforts to improve genetic quality require the availability of superior seeds with massive distribution. This can be done through AI technology using liquid semen and/or frozen semen. One of the factors that determines the success of AI technology in the field is the quality of the liquid or frozen semen used. Liquid semen technology and semen freezing are principally for the efficient use of superior males and maintaining sperm viability over a longer period of time]. The use of liquid semen is an alternative for AI, especially in areas where liquid nitrogen is unavailable. The quality and shelf life of liquid cement depends on the type of diluent used and the dilution process. A good diluent can maintain the quality of spermatozoa during storage and is able to produce high conception rates in the field. Semen quality is one of the main factors in the success of AI. Semen quality can be observed by conducting a semen evaluation both macroscopically (volume, color, odor, consistency and pH) and microscopically (mass movement, motility, abnormalities and concentration). Sperm quality is influenced by age, feed quality, body weight, condition and breed of livestock. Narud *et al*., 2022 also stated that the quality of semen possessed by bull varies. This can be related to factors that influence semen quality. The aim of this study was to determine the quality of post-thawing Donggala bull semen in Andromed and CEP-2 diluents with the addition of egg yolk in different level.

ISSN: 2090-6277/2090-6269/

Materials and methods

The research was carried out at the Central Sulawesi's Regional Technical Implementation Unit (UPTD) for livestock breeding in Sidera, Sigi Regency, Central Sulawesi in 2023.

Material

The study was planned to use a 10 x 5 randomized block design, which included 5 Donggala cattle bulls 7 - 8 ml each as a source of semen and 10 diluent formulas as a treatment. This research used five Donggala bull as semen sources. The diluents used in the semen freezing process were Andromed and CEP-2, with added egg yolk. The freezing process was carried out by placing it above liquid nitrogen vapor for 30 seconds.

Methods

The research used a 10 x 5 randomized block design, which included 5 Donggala cattle bulls as a source of semen and 10 diluent formulas as a treatment. The diluent formula treatments were as follows: A1= Andromedwithout egg yolk, A2= Andromed 95% + egg yolk 5%, A3= AndromeD 90% + egg yolk 10%, A4= Andromed 85% + egg yolk 15%, A5= Andromed 80% + egg yolk 20%, C1= CEP-2 without egg yolk; C2= CEP-2 95% + egg yolk 5%; C3= CEP-2 90% + egg yolk 10% and C4= CEP-2 85% + egg yolk 15% and C5= CEP-2 80% + egg yolk 20%. The variables observed were motility, viability and abnormalities of spermatozoa after freezing.

Semen collection and evaluation

Motility, viability and abnormalities of spermatozoa were observed under a microscope at 400x magnification. Progressive motility of spermatozoa is characterized as spermatozoa that move forward compared to spermatozoa that move in place and do not move expressed as a percentage. Spermatozoa viability was evaluated by eosin staining. Live spermatozoa do not absorb colour, while dead spermatozoa are marked red (absorb colour) expressed in percent. The characteristics of abnormal spermatozoa include damage to both the head and the tail of spermatozoa, as well as the percentage of normal spermatozoa.

Data analysis

The data obtained on the quality of frozen spermatozoa were analyzed for variance using a SPSS statistical program package. The results of the variance analysis showed that there was a significant influence of the diluent formula tested ($P<0.05$), so further tests were carried out following Duncan's test.

Results and Discussion

Fresh Semen Quality

The macroscopic and microscopic quality of fresh semen from 5 Donggala bulls housed with artificial vaginas can be seen in Table 1. The quality of fresh semen from Donggala cattle is almost same as Ongole

Table 1. Average macroscopic and microscopic quality of fresh semen for Donggala bulls.

Quality	Bulls				
	DB1	DB2	DB3	DB4	DB5
Macroscopic					
Volume (ml)	6.14±1.21	6.00±1.91	10.00±1.45	7.44±1.24	7.50±3.54
Colour	Yellowish	Milky white	Milky white	Milky white	Milky white
Consistency	Thick	Thick	Thick	Thick	A bit thick
pH	6.81±0.26	6.50±0.26	6.50±0.00	6.50±0.00	6.75±0.35
Microscopic					
Concentration (x 10^6)	1480.13±309.24	1603.75±300.5	927.29±294.77	1256.67±345.27	1167.50±364.16
Mass movement	2+	≥2+	≥2+	≥2+	2+
Motility	81.25±2.31	79.38±4.17	77.86±4.88	77.78±5.07	72.50±3.54

DB: Donggala Bull

crossbreed cattle. Based on those factors, it is very possible to carry out the freezing process. Good semen quality is dependent on feed maintenance management and genetics. Donggala bulls are fed according to the standard nutritional requirements for bulls. The feed given to beef cattle can be divided into two types, namely forage feed and concentrate feed (Duma *et al*., 2022) Forage is one of the main food sources for beef cattle so that they can meet their needs for life, production and reproduction. There are several types of cut grass, one of which is king grass (Pennisetum purpureum). The grass is a type of grass resulting from a cross between elephant grass (Pennisetum purpureum) and Bengal grass (Pennisetum typhoides). King grass has the advantages of easy to be cultivated and have a high production. Concentrate feed has nutritional content that is composed of several feed ingredients with balanced amounts and contents. Beef cattle certainly need concentrate as it contains propionic acid which is used for the growth of beef cattle. The role of concentrate feed is to increase low nutritional value in order to meet the normal needs of livestock for healthy growth and development (Tahuk *et al*., 2020)

Quality of Frozen Semen in Andromed Medium

The quality of semen after thawing using Andromed diluent added with various levels of egg yolk can be seen in Table 2. Based on the results of analysis of spermatozoa motility using Andromed diluent with the addition of various levels of egg yolk, it showed very significant differences. The best motility, viability and abnormalities of spermatozoa after thawing were demonstrated when using Andromed diluent with the addition of 15% egg yolk. Andromed is a ready-to-use diluent, which is composed of substances needed by spermatozoa during processing, storage at low temperatures, and during the cryopreservation process. According to Bebas *et al*. 2023. Chemical composition of the Andromed diluent is composed of several ingredients needed by spermatozoa during the freezing process, including sodium and potassium which play a role in maintaining the functional integrity of the spermatozoa plasma membrane. Potassium also plays a role in inducing spermatozoa motility, hyperactivation, and can influence spermatozoa survival. The glycerol content in Andromed diluent can increase the resistance of spermatozoa.

Glycerol helps spermatozoa survive temperature drops, thereby reducing sperm damage due to cold shock (Pratiwi et.al., 2014). Egg yolk plays a role in containing lipoproteins and lecithin which protect and maintain the integrity of the protein coat on the cell membrane of sperm to prevent cold shock (Gitayana *et al*., 2023). Fructose functions are as an energy source that is ready to be used in metabolism and maintains osmotic pressure in solvents. The addition of glycerol to the essential diluent for freezing and for unfrozen semen can increase the viability of spermatozoa (Papa, 2015).

Table 2. Semen quality after thawing in Andromed diluent.

Diluent	Quality of frozen semen[1]		
	Motility (%)	Viability (%)	Abnormality (%)
PA1	41.77±4.05^{e}	65.91±3.27^{de}	20.91±0.56^{d}
PA2	47.44±5.74^{cd}	69.13±3.47^{c}	20.04±0.95^{cd}
PA3	51.14±4.70^{bc}	72.90±2.03^{b}	19.48±2.18^{bcd}
PA4	58.68±4.46^{a}	76.96±3.01^{a}	17.07±0.46^{a}
PA5	52.57±6.68^{b}	73.42±2.63^{b}	18.16±1.59^{ab}

Data are expressed as Mean±SD.
[1]Different superscript in column showed significantly different (P<0.01)

Quality of Frozen Semen in CEP Diluent

The quality of frozen semen in CEP-2 diluent can be seen in Table 3. Semen quality including motility, viability and abnormalities in CEP-2 diluent showed very significant differences. The best quality was shown with values of 44.77±3.71, 67.48±2.22, 18.65±0.58 respectively for motility, viability and abnormalities in the CEP-2 diluent which was added with 15% egg yolk. Caudal Epididymal Plasma (CEP-2) is a diluent that has almost the same ionic composition as bovine epididymal cauda fluid that is developed by Veberckmoes *et al*. (2004). CEP-2 has an ion composition, pH and osmolarity that mimic the conditions of bovine cauda epididymal plasma (Veberckmoes *et al*., 2004). The CEP-2 diluent is adapted to the conditions of the epididymis in a male reproductive tract both in terms of ion composition, pH and osmolarity. Semen diluent still requires macromolecules such as egg yolk which has the function of protecting spermatozoa during storage at cold temperatures. Ismaya (2014) stated that egg yolk can be used as a macromolecular ingredient in diluents because it is able to protect the perfection of the protective colloids for spermatozoa. Bergeron and Puttaswamy (2006) explained that the main components of egg yolk which provide protection for spermatozoa during storage include low density lipoprotein (LDL), phospholipids and cholesterol. LDL can bind to detrimental factors from bovine seminal plasma so that it can prevent the loss of membrane lipids and cholesterol. Zeron *et al.* (2002) phospholipids from egg yolk are able to associate with spermatozoa membranes. Therefore, phospholipids play a role in the lipid phase transition (liquid phase to gel phase) during temperature changes, thereby reducing sensitivity to cold temperatures. Meanwhile, cholesterol from egg yolk can increase the viability of spermatozoa after the freezing process.

Table 3. Quality of semen after thawing in CEP-2 (Cauda Epididymis Plasma) diluent.

Diluent	Quality of frozen semen[1]		
	Motility (%)	Viability (%)	Abnormality (%)
PA1	21.71±3.23^{h}	58.56±2.68^{g}	22.35±1.22^{e}
PA2	28.76±4.74^{g}	62.45±1.96^{f}	21.86±1.08^{e}
PA3	44.77±3.71^{de}	67.48±2.22^{cd}	18.65±0.58^{bc}
PA4	42.35±4.25^{de}	63.88±3.01^{ef}	19.89±1.73^{cde}
PA5	36.12±2.14^{f}	63.12±1.72^{f}	19.58±1.42^{bcd}

Data are expressed as Mean±SD.
[1]Different superscript in column showed significantly different (P<0.01)

Conclusion

Andromed and CEP-2 diluents with the addition of egg yolk could be used as a cryopreservation of donggala bull sperm, an effort to preserve local genetic source at central Sulawesi.

Acknowledgements

National Research Agency (BRIN) for supporting financial research. LPDP has provided financial support for this research Livestock Breeding (UPTD) for the research site facilities.

Conflict of interest

The authors have no conflict of interest to declare.

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