The Effect of Different Types of Commercial Diluents on the Semen Quality of *Polled* Bali Bull

Andi Rezda Sri Mulyani[1], Ronny Markus Jami Riwu[2], Lutfiah Aminah[3], Siti Nuraisyah Hamsir[3], Sudirman Baco[3], Herry Sonjaya[3], Sri Gustina[3] and Hasbi Hasbi[3*]

[1]Student of Animal Science, Department of Animal Breeding and Reproduction, Faculty of Animal Science, Universitas Brawijaya, Malang 65145, Indonesia
[2]Department of Animal Science, Faculty of Animal Husbandry, Marine and Fisheries, Nusa Cendana University, Kupang 85228, Indonesia.
[3]Department of Animal Production, Faculty of Animal Science, Hasanuddin University, Makassar, 90245, Indonesia

Abstract. Polled Bali cattle are a variant of Bali cattle characterized by the natural absence of horn growth. In artificial insemination programs, Andromed® is a commonly used commercial semen diluent; however, limited studies have evaluated its performance compared to other diluents containing lecithin-based components. This study aimed to assess the semen quality of polled Bali bull using three commercial diluents: Andromed®, Steridyl®, and Bovifree®. The research was conducted at the UPT-PIBPS, Livestock and Animal Health Service of South Sulawesi Province, and the In Vitro Embryo Production Laboratory, Hasanuddin University. Semen was collected from two polled Bali bulls aged 5 and 7 years. A completely randomized design (CRD) was employed with three treatment groups and seven replications. Semen quality was evaluated based on progressive motility, viability, abnormalities, and Intact Plasma Membrane (IPM). Data were analyzed using analysis of variance (ANOVA) followed by Duncan's multiple range test. The results indicated that Andromed® significantly improved sperm motility ($P<0.01$) compared to Steridyl® and Bovifree®. Viability did not differ significantly among the diluents ($P>0.05$). Sperm abnormalities were significantly higher in the Steridyl® group ($P<0.01$), while Andromed® showed significantly better intact plasma membrane than Steridyl® ($P<0.05$), but was not substantially different from Bovifree® ($P>0.05$). In conclusion, Andromed® demonstrated better performance in most semen quality parameters compared to Steridyl® and Bovifree® for polled Bali bulls semen cryopreservation.

Keywords: Commercial diluent, Frozen semen, Polled Bali bull, Semen quality.

1 Introduction

Indonesia has considerable potential for livestock farming due to optimal conditions for raising various types of livestock, including cattle. Among these, Bali cattle are one of the most widely developed breeds. Bali cattle have been designated as an indigenous breed of Indonesia through the Minister of Agriculture Decree No. 325/kpts/OT.140/1/2010 and are registered in the Domestic Animal Diversity Information System of the Food and Agriculture Organization (DAD-IS FAO) [1]. In South Sulawesi, Bali cattle are developed in both horned and polled varieties, the latter known as Polled Bali cattle. Polled Bali cattle are Bali cattle

*Corresponding author: hasbi_fapetunhas@yahoo.com

whose horns do not develop naturally. Despite lacking horns, Polled Bali cattle's body dimensions and characteristics are identical to those of horned Bali cattle [2]. They are widely promoted because of their economic and management advantages, including reducing the cost of horn removal, lowering the risk of injury and infection caused by horn friction, and minimizing livestock stress during dehorning. Therefore, Polled Bali cattle are widely developed by communities due to their benefits.

One of the main strategies to maintain and increase the population of Polled Bali cattle is using Artificial Insemination (AI) technology. In general, the semen commonly used for AI is frozen semen, but its quality will decrease by 50% from fresh semen during the freezing and thawing process. The use of diluents serves to maintain semen quality during freezing and thawing so that it remains optimal and can support successful fertilization [3]. The diluent that should be used is one that is inexpensive, simple, practical, and has a high level of protection for sperm. Various diluents have been used to dilute semen, one of which is a commercial diluent. Commercial diluents meet the basic requirements for diluent use, which are easy to use, simpler preparation, and contain ingredients that can maintain spermatozoa quality.

The use of commercial diluents with a lecithin-based formulation has been widely adopted; however, their effectiveness must be specifically evaluated for each species and breed, as each species, breed, and even individual livestock can respond differently to the same type of diluent. A previous study that compared three different types of diluents with a lecithin-based ingredient (Andromed®, Steridyl®, and Bovifree®) in Toraya buffalo showed no significant difference in maintaining frozen semen quality, with PTM motility ranging from 45.93% to 47.09%, viability of 56.21%-60.27%, and intact plasma membrane of 57.73%-61.36% [4]. Another study comparing commercial diluents, which are Andromed®, Sterydil®, and OptiXcell® in ram, reported that the use of Sterydil and OptiXcell diluents showed better motility, viability, and recovery rates compared to Andromed [5].

Despite these findings, there remains a significant research gap regarding the evaluation of commercial lecithin-based diluents such as Andromed® (soybean lecithin), Steridyl® (sterilized egg yolk lecithin), and Bovifree® (synthetic liposome lecithin) in Bali cattle, particularly polled Bali cattle, which are local cattle with specific genetic characteristics. Therefore, further studies are needed to determine the quality of Bali polled cattle semen using a commercial diluent with lecithin-based sources. This study aims to compare three commercial diluents—Andromed®, Steridyl®, and Bovifree®— on the semen quality of Polled Bali bulls to identify potential alternatives for the cryopreservation of Bali cattle semen, particularly Polled Bali cattle. The quality parameters include motility, viability, abnormalities, and intact plasma membrane (IPM). The results of this study are expected to provide information on the quality of Polled Bali cattle semen using Andromed®, Steridyl®, and Bovifree® diluents.

2 Materials and methods

2.1 Materials

This study was conducted at the Technical Implementation Unit for Regional Artificial Insemination Center (RAIC) of the South Sulawesi Provincial Livestock and Animal Health Office, in Pucak Village, Tompobulu Sub-district, Maros District, South Sulawesi Province, Indonesia for the collection of Polled Bali cattle semen and the semen freezing process. Quality testing of frozen Polled Bali bulls semen was conducted at the In Vitro Embryo Production Laboratory, Research and Community Service Institute, Hasanuddin University.

The materials used in this study included three commercial diluents with the brand names Andromed®, Steridyl®, and Bovifree®, Aquabidest, eosin and negrosin, HOS solution, 0.25 mL ministraw, pH special indicator paper, and physiological NaCl. The equipment used in this study included a trinocular microscope set, an artificial vagina set, a filling and sealing machine set, a cold handling cabinet (4–5°C), scissors, a photometer, collection tubes, a heating table (37°C), cryocontainers, object glass, and cover glass.

2.2 Methods

2.2.1 Preparation of diluents

The diluents used in this study are commercial diluents, namely Andromed®, Steridyl®, and Bovifree®, each mixed with aquabidest in the following ratios: Andromed® 1:4, Steridyl® 1:1.5, and Bovifree® 1:4 (these ratios are following the procedures stated on the packaging). The diluents are then homogenized and placed in a water bath at 35°C until the dilution process is performed.

2.2.2 Semen collection and processing

Semen collection is performed once a week in the morning. The semen collection process uses an artificial vagina by the bull master from the Artificial Insemination and Semen Production Service Unit (UPT-PIBPS) of the South Sulawesi Province Livestock and Animal Health Service. The semen is then taken to the laboratory for macroscopic and microscopic evaluation.

Fresh semen that has been evaluated is diluted according to the treatment and then frozen. 1.2 ml of semen is divided into three samples and then diluted with Andromed®, Steridyl®, and Bovifree® using an insemination dose of 25 million cells in 0.25 ml per straw. The volume of diluent used is calculated mathematically using the formula:

$$volume\ total(mL) = \frac{V \times K \times M}{(25 \times 10^6)} \times 0{,}25$$

Description:
V: Volume of semen (mL)
K: Sperm concentration (106/mL)
M: Sperm motility (%)

Semen mixed with diluent is then homogenized slowly and then packaged into 0.25 mL ministraws using an automatic machine. After packaging, the straws are arranged on a freezing rack (freezing machine) and then equilibrated at a temperature of 4–5°C for three hours. For further testing, frozen semen is stored in a container filled with liquid nitrogen.

2.2.3 Semen evaluation

Macroscopic evaluation refers to the direct and uncomplicated assessment of semen based on observable parameters such as volume, smell, color, pH, and consistency.

Microscopic evaluation includes mass motility, individual motility, sperm concentration, viability, sperm morphology, and an intact plasma membrane.

2.2.4 Statistical Analysis

The data were analyzed using analysis of variance (ANOVA) with a completely randomized design (CRD) consisting of three treatments using different dilutions (Andromed®, Steridyl®,

and Bovifree®) and seven collections as replicates. Treatments that obtained significantly different results were followed up with Duncan's test as a further test.

3 Results and discussion

3.1 Quality of Fresh Semen from Polled Bali Bulls

Fresh semen that has been collected is evaluated as an indicator of semen that can be processed further. The results of the evaluation of fresh semen from Polled Bali bulls can be seen in Table 1.

Table 1 Macroscopic and microscopic quality of fresh semen from Polled Bali Bulls.

Parameters	Value (Mean±SD)
Macroscopic Evaluation	
Color	Creamy
pH	6.4 ± 0.00
Smell	Distinctive
Volume (ml)	5.77 ± 1.64
Microscopic Evaluation	
Concentration (cell/ml)	$848 \times 10^6 \pm 92 \times 10^6$
Motility (%)	70.00 ± 0.00

Table 1 shows that the semen of Polled Bali bull is cream-colored, indicating that the semen of Polled Bali bull observed is normal. The same study reported that the color of bovine semen ranges from milky white to cream. The color of bovine semen can be clear, milky white to cream due to several factors such as feed quality, frequency of ejaculation, and the concentration and consistency of the semen [6].

The pH value of Polled Bali bull semen evaluated in this study was 6.4, indicating that the pH of Polled Bali bull semen in this study was normal. A previous study reported that the normal acidity level (pH) for various cattle breeds ranges from 6 to 7. Differences in semen pH are influenced by the condition of the bull and the citric acid content in the semen [7]. The viability of semen can be assessed based on the pH of the evaluated semen.

The evaluated Polled Bali bull semen has a characteristic semen, indicating that the evaluated semen is normal and not contaminated by other substances. Semen has a distinctive smell that indicates that it is of good quality and not contaminated with bacteria. Semen that does not have a distinctive smell, like livestock itself, can be affected by contamination from urine, feces, and sweat, causing it to have a pathological smell [8].

The semen volume of polled Bali bull in this study averaged 5.77 ml, which is still within the normal range. Variations in semen volume can be caused by various factors such as age, physical condition, body weight, environment, and male libido. In addition, insufficient lighting for livestock can reduce semen volume, as it can inhibit FSH hormone production [1].

Microscopic evaluation results showed an average concentration of fresh semen from Polled Bali bull of 848×10^6 cells/ml, indicating that the evaluated semen can be processed into frozen semen. This result is lower than previous studies, which found an average concentration of fresh Bali bull semen of 1,646.60 million/ml. Semen concentration can be influenced by the physiological condition of the animal, frequency of ejaculation, testicle size, and environment [8]

Semen motility in the study had an average of 70%, indicating that the evaluated semen was normal and could be processed further. Fresh semen motility must meet the requirement

of 70% to be processed further. The diversity of motility values is influenced by the season. Motility values can be influenced by storage methods, age, and storage frequency. The results of macroscopic and microscopic evaluations show that fresh semen from polled Bali cattle can proceed to the next process, which is freezing [9]. Macroscopic and microscopic evaluations indicate that fresh semen from Polled Bali bulls meets the requirements for the freezing process.

3.2 Quality of Frozen Polled Bali Bull Semen Using Commercial Dilutant

Fresh semen that has undergone evaluation and obtained satisfactory results can then proceed to the freezing process. Before freezing, semen that has passed evaluation is first diluted with nutrients for the semen during the freezing process so that semen quality can be maintained. Microscopic evaluation can be seen in Table 2.

Table 2 Microscopic Quality of Frozen Polled Bali Bull Semen Using Commercial Diluent

Diluents	Parameter			
	Motility(%)**	**Viability(%)**	**Abnormality(%)****	**IPM(%)***
Andromed®	48.57 ± 3.77[a]	85.65 ± 5.04	11.56 ± 1.65[b]	82.16 ± 2.71[a]
Steridyl®	32.85 ± 4.87[b]	80.18 ± 6.19	16.97 ± 1.42[a]	71.47 ± 9.12[b]
Bovifree®	33.57 ± 4.75[b]	82.60 ± 8.87	12.40 ± 2.05[b]	76.66 ± 3.57[ab]

Notes:
* Different superscripts in the same column indicate significant differences (P<0.05)
** Different superscripts in the same column indicate highly significant differences (P<0.01)

3.2.1 Sperm Motility

Sperm motility is one of the parameters used to determine the ability of sperm to move toward the egg. The results in Table 2 show that different diluents have a highly significant effect on the motility of frozen semen from Polled Bali bull. Statistical analysis indicates that Andromed® is significantly higher (P<0.01) compared to Steridyl® and Bovifree®, while Steridyl® and Bovifree® are not significantly different (P>0.05). This indicates that the Andromed® diluent is more effective in maintaining sperm motility compared to the Steridyl® and Bovifree® diluents. According to the National Standards Agency 4869.1:2017, semen motility above 40% meets the criteria for insemination, so it can be concluded that in this study, only the Andromed® diluent meets the requirements for insemination.

The fructose, glycerol, citric acid, and antioxidant content in the Andromed® diluent can provide energy to sperm and maintain sperm viability during the freezing process. The fructose content in the Andromed® diluent, which functions as an energy producer in the form of ATP, is beneficial for sperm movement, protects sperm membrane integrity, and maintains calcium ion balance, thereby preserving sperm motility from the dilution process to the freezing process [10].

The low motility levels in Steridyl® and Bovifree® diluents are likely due to the absence of antioxidants that protect the plasma membrane, thereby failing to maintain frozen semen motility during prolonged storage. The presence of antioxidants, whether from seminal plasma or external addition, plays a role in maintaining sperm quality by neutralizing excess Reactive Oxygen Species (ROS) during the freezing process [11].

3.2.2 Sperm Viability

Viability is one of the determining factors for the success of artificial insemination. Viability evaluation is performed using eosin-negrosin staining. Dead sperm are marked by pink-

colored heads, caused by damage to the cell membrane, which absorbs the eosin-negrosin solution.

Table 2 shows that different diluents did not significantly affect (P>0.05) the viability parameters. The average results for Andromed®, Steridyl®, and Bovifree® were 85.65%, 80.18%, and 82.60%, respectively. The thawing process causes a decrease in sperm quality, including viability, due to changes in temperature and metabolic changes within the cells, which result in damage to the sperm membranes [12]. The analysis results show that the viability of Andromed® diluent is higher (with an average of 85.65%) than the other two diluents. However, all three diluents used show viability above 80%, which means that semen using these three diluents can be used for AI.

3.2.3 Sperm Abnormalities

Sperm abnormalities are conditions in which sperm have abnormal shapes, either in the head or tail regions. Abnormalities can be evaluated using eosin-negrosin staining. The abnormalities observed in this study include detached head and tail, double head, microcephalic (small head), round head, curled tail, and short tail. Abnormalities are classified into primary, secondary, and tertiary abnormalities. Secondary abnormalities occur when there are cytoplasmic droplets in the midpiece of the tail. Tertiary abnormalities are abnormalities in the tail [13].

The results in Table 2 indicate that different diluents have a significant effect on the abnormalities of frozen semen from Polled Bali bull. Statistical analysis shows that Steridyl® is significantly higher (P<0.01) compared to Andromed® and Bovifree®, but Andromed® and Bovifree® are not different (P>0.05). The abnormality values of the three treatments were below 20%, according to the regulations for frozen semen circulation regarding abnormality values. According to the Ministry of Agriculture Regulation No. 10 of 2016, semen from superior bull used for producing frozen semen must have an abnormality percentage of ≤20%.

The different abnormality values in each diluent are due to the phospholipid content in Andromed® and Bovifree® diluents, which can maintain abnormality better than Steridyl®. Differences in the composition of each diluent can provide varying levels of protection against spermatozoa abnormality. The level of abnormality in spermatozoa can be caused by various factors such as temperature, environmental factors, and handling factors during storage [14].

3.2.4 Intact Plasma Membrane (IPM)

The integrity of the plasma membrane is an important parameter for sperm because the plasma membrane functions to protect organelles from mechanical damage that may occur. The evaluation of intact plasma membranes was performed using HOS solution, where sperm with intact plasma membranes were marked by curved tails, and sperm with damaged plasma membranes were marked by straight tails.

Table 2 shows that different diluents have a significant effect on the Intact Plasma Membrane (IPM). Statistical analysis indicates that Andromed® is significantly higher (P<0.05) compared to Steridyl®, but not different from Bovifree® (P>0.05), while Steridyl® and Bovifree® are not different (P>0.05). The quality of IPM in the three diluents is suitable for use in artificial insemination, with Andromed® (82.16%), Steridyl® (71.47%), and Bovifree® (76.66%), although the IPM value of Steridyl® is lower than the other two diluents. Previous studies have shown that there is no difference in the integrity of sperm plasma membranes when using three different diluents (Andromed®, Steridyl®, and Bovifree®) in buffalo Toraya. During the freezing and thawing process, extreme temperatures can damage

cell membranes; therefore, sperm diluents must contain ingredients that can protect cell membranes [4].

The ingredients contained in the diluent have their own roles in maintaining spermatozoa quality. The diluent used in this study contains vegetable lecithin (Andromed®), sterile egg yolk (Steridyl®) (Minitube), and phospholipids (Bovifree®) (Minitube), which are believed to be able to maintain the integrity of the plasma membrane so that the plasma membrane does not experience a drastic decline. The lipoprotein and phospholipid content protects the plasma membrane by maintaining osmotic pressure and reducing damage to spermatozoa membranes from cold shock. [15].

4 Conclusion

From the results of this study, it can be concluded that Andromed® diluent provides superior semen quality in terms of motility, viability, abnormality, and intact plasma membrane, compared to Bovifree® and Steridyl®. Considering the semen quality standards for artificial insemination, Andromed® is the most suitable diluent for cryopreservation of Polled Bali bull semen. Meanwhile, Bovifree® and Steridyl® are not yet recommended for insemination use, and further research is needed to improve their effectiveness or develop alternative formulations.

This research was supported by the RIIM LPDP grant number 35/IV/KS/06/2022

References

1. Saputra D. J, Ihsan M. N, and Isnaini N. (2017). Korelasi Antara Lingkar Skrotum Dengan Volume Semen, Konsentrasi dan Motilitas Sperm Pejantan Sapi Bali. Journal of Tropical Animal Production, 18(2), 59–68. DOI:doi.org/10.21776/ub.jtapro.2017.018.02.9
2. Hasbi H, Sonjaya H, Baco S, Amalia R, and Gustina S. (2021). Characteristics of Libido and Testosterone Concentration of Polled and Horned Bali Bulls after GnRH Injection, 26(3), 108-114. DOI:10.14334/jitv.v26i3.2851
3. Tethool A. N, Ciptadi G, Wahjuningsih S, and Susilawati T. (2022). Bali Cattle Semen Characteristics and Diluent Types: A Review. Journal of Tropical Animal and Veterinary Science, 12(1), 45–57. DOI:10.46549/jipvet.v12i1.214
4. Riwu, R. M. J., Arifiantini, R. I., dan Karja, N. W. K. (2023). Comparison of Different Lecithin Diluent for Cryopreservation of Toraya Buffalo semen. Tropical Animal Science Journal, 46(4), 396-402. DOI: https://doi.org/10.5398/tasj.2023.46.4.396
5. Setiadi, D. R., Magfira, Riwu, R. M. J., and Arifiantini, R. I. (2025). Effectiveness of Various Commercial Extenders in Cryopreserving Semen of Local Indonesian Rams. Jurnal Bioteknologi & Biosains Indonesia. 12(1), 68-77. DOI:doi.org/10.55981/jbbi.2025.9208
6. Aisah, S., N. Isnaini, dan S. Wahyuningsih. (20170. Kualitas semen segar dan *recovery rate* sapi Bali pada musim yang berbeda. Jurnal Ilmu-Ilmu Peternakan, 27(1). 63-79. DOI: doi.org/10.21776/ub.jiip.2017.027.01.06
7. Yendraliza Y, Abadi H, Misrianti R, Ali A, and Effendi A. (2019). Body Size Identification and Semen Quality of Kuantan Cattle. Jurnal Ilmiah Peternakan terpadu, 7(1), 186–91. DOI:doi.org/10.23960/jipt.v7i1.p186-191
8. Wijayanti A, Suprayogi T. W, Prastiya R. A, Hernawati T, Sardjito T, Saputro AL, et al. (2023). Effect of Addition of Green Tea Extract (Camellia sinensis) in Egg Yolk Tris

Diluter on Sperm Quality in Bali Cattle (Bos sondaicus) After Freezing. Jurnal Medik Veteriner, 6(1), 66–74. DOI:10.20473/jmv.vol6.iss1.2023.66-74

9. Komariah, Arifiantini R. I, Aun M, and Sukmawati E. (2020). Kualitas semen segar dan produksi semen beku sapi pejantan madura pada musim yang berbeda. Jurnal Ilmu Produksi dan Teknologi Hasil Peternakan, 8(1):15–21. DOI : doi.org/10.29244/jipthp.8.1.15-21
10. Mukhlis M, Dasrul D, and Sugito S. (2017). Analysis of Aceh Cattle sperm motility after freezing using Andromed® with different concentration. Jurnal Agripet, 17(2), 112–120. DOI: doi.org/10.17969/agripet.v17i2.8373
11. Atiken R. J, and Drevet J. R. (2020). The importance of oxidative stress in determining the functionality of mammalian sperm: A two-edged sword. Antioxidants. 9(111), 1-19. DOI: 10.3390/antiox9020111
12. Susilawati, T., Ihsan, M. N., Wahjuningsih, S., Isnaini, N., Rachmawati, A., Yekti, A. P. A., and Utami, P. (2022). Manajemen Reproduksi dan Inseminasi Buatan. Universitas Brawijaya Press. Malang
13. Susilawati T. (2011). Spermatology. Universitas Brawijaya Press. Malang.
14. Seuk, M. O. (2018). Pengaruh Frekuensi Penampungan terhadap Kualitas Sperm Sapi Bali. Journal of Animal Science, 3(4), 51–53. DOI:10.32938/ja.v3i4.540
15. Kusumawati, E. D. (2022). Sexing Spermatozoa Pada Kambing. Media Nusa Creative (MNC Publishing). Malang