Development of a frozen bovine semen diluent: comparison of particle size of commercial and home-made diluents

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Abstract. A variety of diluents, both homemade and commercial, are used in Indonesian artificial insemination centers to freeze bovine semen. One of the homemade diluents used is Tris egg yolk. The egg yolk is used as a source of lecithin to protect the sperm membrane during freezing. The smaller the lecithin particles, the more effectively they can bind to the cell membrane. In addition, the particle size facilitates the analysis of sperm quality using Computer Assisted Sperm Analysis (CASA). This study aimed to perform particle analysis of lecithin in homemade and commercial diluents using a particle size analyzer (PSA). The diluents used in this study were Tris egg yolk, Andromed (Minitube, Germany), Bovifree (Minitube, Germany), OptiXcell (IMV, France), and BioXcell (IMV, France). The results showed that the Tris egg yolk diluent had the widest particle size range, from 584.5 ± 2.2 to 3255.7 ± 48.84 nm. The smallest commercial diluent was OptiXcell (64.6 ± 2 nm), followed by BioXcell (186.2 ± 2.2 nm), Andromed (245.7 ± 2.8 nm) and Bovifree (278.5 ± 2.5 nm). The sperm kinematics of frozen semen in Tris egg yolk diluent were challenging to analyze. In contrast, the kinematics of semen in commercial dilutions could be visualized and evaluated according to the established criteria. This study's results indicate that the commercial diluent's particle size is smaller than that of the homemade diluent and does not affect the results of the CASA assessment. Consequently, developing homemade diluents with a particle size comparable to commercial diluents is essential.

1 Introduction

Indonesia has several Artificial Insemination Centres (AICs) spread over different islands [1]. AICs produce frozen semen from superior bulls for use in artificial insemination (AI). A variety of diluents, both homemade and commercial, are used in Indonesian AI centers

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to freeze bovine semen. One of the homemade diluents used is Tris egg yolk. The egg yolk is used as a source of lecithin to protect the sperm membrane during freezing. The disadvantages of using egg yolk in semen cryopreservation are that this animal-derived component has potential microbiological risks (including disease) [2] and inconsistent standardization of its biochemical composition [3]. Commercial diluents include Andromed (Minitube, Germany), Bovifree (Minitube, Germany), OptiXcell (IMV, France), and BioXcell (IMV, France), which are free of animal protein. Andromed (Minitube, Germany), Bovifree (Minitube, Germany), and BioXcell (IMV, France) contain soya lecithin (phospholipids) and Tris buffer, while OptiXcell (IMV, France) includes liposomes [4]. Andromed consists of buffer (tris-(hydroxymethyl)-aminomethane, citric acid), sugar (fructose), antioxidants, glycerol, water and four types of antibiotics (tylosin tartrate, gentamicin sulfate, spectinomycin and lincomycin). OptiXcell (IMV, France) diluents are made from the same ingredients but contain liposomes. The four diluents have the same composition and characteristics, maintaining a pH between 6.8 and 7.2. They also contain antioxidants to reduce oxidative stress and cold shock [5]. The advantages of commercial diluents are industrially standardized and include the absence of animal protein, long shelf life, ease of use, and sterilization. The diluent is transparent, and the particles are homogeneous in size, making it easier to assess semen quality. The technology of commercial diluents needs to be carried out to improve the technology of making homemade diluents in Indonesia. The smaller the lecithin particles, the more effectively they can bind to the cell membrane. In addition, particle size facilitates the analysis of sperm quality using Computer Assisted Sperm Analysis (CASA). This study aimed to perform particle analysis of lecithin in homemade and commercial diluents using a particle size analyzer (PSA).

2 Materials and methods

2.1 Preparation of homemade and commercial diluents

The diluents used in this study were Tris egg yolk, Andromed (Minitube, Germany), Bovifree (Minitube, Germany), OptiXcell (IMV, France), and BioXcell (IMV, France). The homemade diluent uses Tris diluent by adding 20% (v/v) egg yolk. The composition of the Tris diluent consists of Tris-hydroxymethyl-aminomethane 3.09% (w/v) (Merck, Germany), citric acid 1.73% (w/v) (Merck, Germany), fructose 1.27% (w/v) (Merck, Germany), gentamicin 500 µg/ml (Sigma-Aldrich) and glycerol 6% (v/v) (Merck, Germany) in sterile deionized water. Commercial diluents were used by adding sterile deionized water in the following proportions: Andromed (Minitube, Germany) = 1:4, Bovifree (Minitube, Germany) = 1:4, OptiXcell (IMV, France) = 1:2 and BioXcell (IMV, France) = 1:4.

2.2 Particle size evaluation with CASA

The study was conducted to determine the size of the diluent particles that can be detected by the movement pattern parameters using the Computer Assisted Sperm Analyzer (CASA) system (SpermVision Minitube Germany). A 5-8 microlitre diluent

sample was placed on a glass slide, covered with a coverslip, and then observed using CASA.

2.3 Particle size analyser (PSA)

The study was conducted to determine the size of the diluent particles using a particle size analyzer (PSA) with Horiba SZ-100. Particle size analysis is performed by dynamic light scattering (DLS). Depending on the sample's physical properties, the dynamic range is 0.3 nm—8 μm.

2.4 Data analysis

Quantitative data were analyzed using ANOVA (analysis of variance), followed by Duncan's test to determine significant differences between treatments. Analyses were performed using SPSS IBM 25 and MS Office Excel software.

3 Results and discussion

3.1 Result

The results of particle evaluation in commercial diluents Andromed (Minitube, Germany), Bovifree (Minitube, Germany), OptiXcell (IMV, France), and BioXcell (IMV, France) macroscopically and CASA kinematics under a microscope at 200x magnification are shown in Figure 1. Particles in commercial diluents do not affect the results of the CASA evaluations.

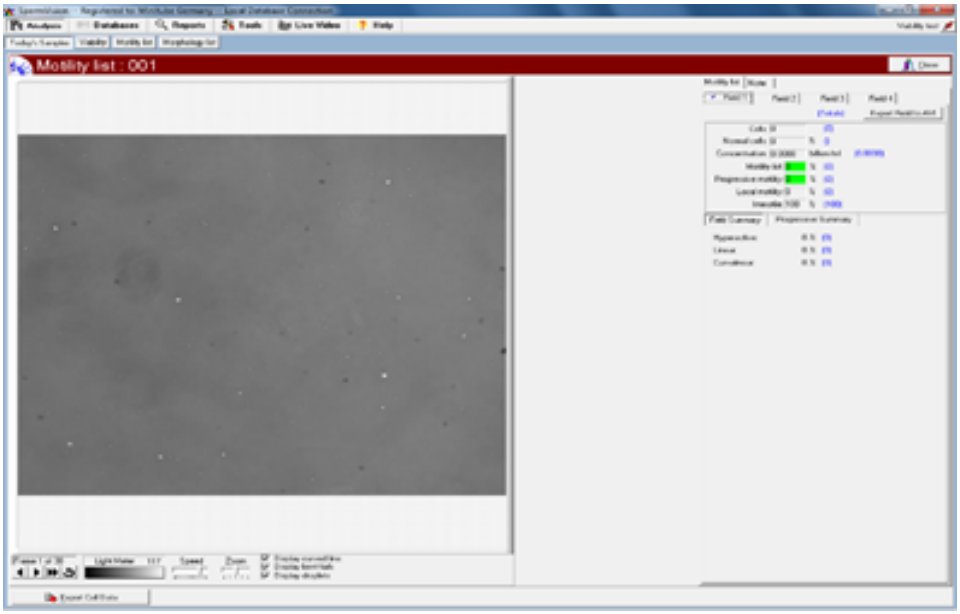

Fig. 1. Evaluation of commercial diluents using CASA.

Figure 2 shows the results of the evaluation of particles in Tris egg yolk diluent macroscopically and CASA kinematics under a microscope with a magnification of 200 times. CASA can detect particles in Tris Egg Yolk diluent.

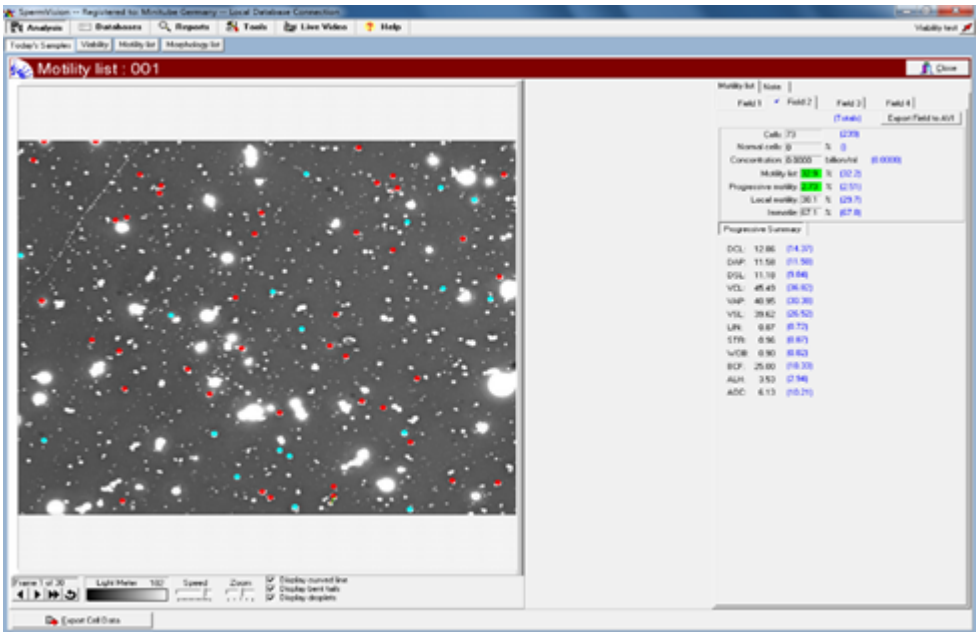

Fig. 2. Evaluation of Tris egg yolk diluent using CASA.

The study was conducted to determine the size of the diluent particles using a particle size analyzer (PSA) in Figur 3. The particle size of the commercial diluent is smaller than that of the homemade diluent.

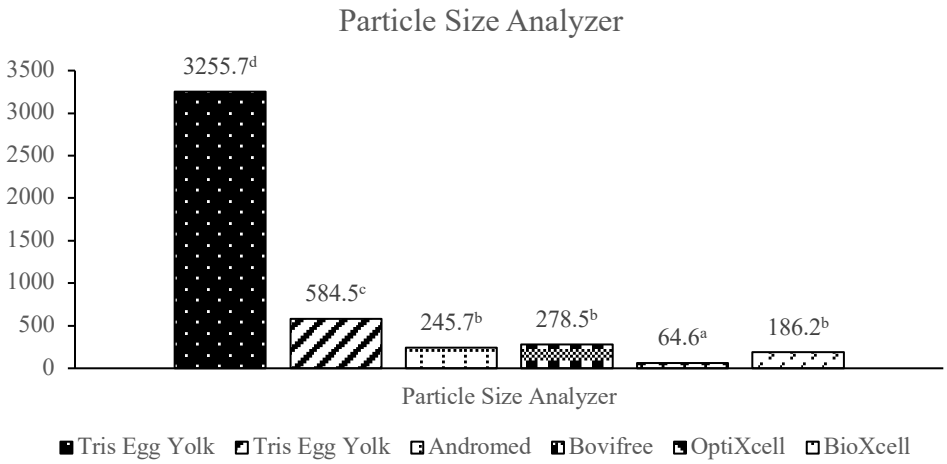

Fig. 3. PSA results of homemade and commercial diluents.

3.2 Discussion

Studying particle size in diluents is essential for developing homemade diluents in Indonesia. Homemade diluents have been used since 1976 with the establishment of the Artificial Insemination Centre in Indonesia. The development of diluents began with commercial diluents in Indonesia in 2000. Andromed and Bovifree diluents are manufactured by Minitube Germany with phospholipid content as a substitute for egg yolk [6]. BioXcell and OptiXcell diluents are manufactured by IMV France with phosphatidylcholine and liposomes replacing egg yolk [7]. Commercial diluents are easy to use, free from microbiological contamination, and have a long shelf life. Observation with CASA provides an accurate assessment. The results of particle size evaluation using CASA on commercial diluents are increasingly clear without the need for kinematic parameter software. TKT diluents analyzed with CASA contain egg yolk particles the size of spermatozoa, which may interfere with the assessment of motile spermatozoa, and the results are less accurate [8]. Based on the results of PSA measurements on Tris egg yolk diluent, there are two particle sizes, small 584.5 ± 2.2 nm and large 3255.7 ± 48.84 nm. The large particle size in Tris egg yolk diluent, which is one of the homemade diluents, can be detected by CASA. This finding contrasts the commercial diluents, which have the smallest particle size of 64.6 ± 2 nm on OptiXcell and the largest particle size of 278.5 ± 2.5 nm on Bovifree, making them undetectable on CASA. The advantages of commercial diluents are industrially standardized and include the absence of animal protein, long shelf life, ease of use, and sterilization. The medium is transparent, and the particles are homogeneous in size, making it easier to assess the semen quality.

4 Conclusions

The results of this study indicate that the particle size of the commercial diluent is smaller (64.6±2 - 278.5±2.5 nm) than that of the homemade diluent (584.5±2.2 - 3255.7 ± 48.84 nm). The commercial diluents do not affect the results of the CASA evaluation. It is, therefore, evident that developing homemade diluents with a particle size comparable to commercial diluents is essential.

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References

1. J. Jakaria, G.G. Gunara, K. Puja, R.I. Arifiantini, S. Herliantien, B. Bakar, A. Hutasoit, S.H.M. Herwiyati, E. Hajirin, Jamarizal, Y. Maulana, H. Chakra, M. Zuljisman, F. Deflaizar, T.U. Abrianto, S. Hidayati, Roadmap swasembada pejantan unggul 2018-2022. Direktorat Perbibitan dan Produksi Ternak, Direktorat Jenderal Peternakan dan Kesehatan Hewan, Kementerian Pertanian, Jakarta (ID), 2018.

2. S. Chelucci, V. Pasciu, S. Succu, D. Addis, G.G. Leoni, M.E. Manca, F. Berlinguer, Soybean lecithin–based extender preserves spermatozoa membrane integrity and fertilizing potential during goat semen cryopreservation. Theriogenology. **83**, 1064-1074 (2015). https://doi.org/10.1016/j.theriogenology.2014.12.012
3. M. Emamverdi, M. Zhandi, A.Z. Shahneh, M. Sharafi, A. Akbari-Sharif, Optimization of ram semen cryopreservation using a chemically defined soybean lecithin-based extender. Reprod Domest Anim. **48**, 899–904 (2013). http://dx.doi.org/10.1111/rda.12183
4. A.A.A. Swelum, I. M. Saadeldin, H. Ba-Awadh, M.G. Al-Mutary, A.F. Moumen, A.N. Alowaimer, H. Abdalla, Efficiency of commercial egg yolk-free and egg yolk-supplemented tris-based extenders for dromedary camel semen cryopreservation. Animals. **9**, 1-15 (2019). https://doi.org/10.3390/ani9110999
5. G.S. Bustani, F.H. Baiee, Semen extenders: an evaluative overview of preservative mechanism of semen and semen extenders. Vet. World. **14**, 12201233 (2021). https://doi.org/10.14202/vetworld.2021.1220-1233
6. Minitube bovine semen extenders, http://www.minitube.com, acsess: 08/2023
7. LIPOSOMES-BASED MEDIUM FOR BOVINE SEMEN, http://www.imv-technologies.com, March 2020 version
8. P. Vincent, S.L. Underwood, C. Dolbec, N. Bouchard, T. Kroetsch, P. Blondin, Bovine semen quality control in artificial insemination centers. Anim Reprod. **9**, 153–65 (2021). https://doi.org/10.1002/9781119602484.CH81