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IOP Conf. Series: Materials Science and Engineering **434** (2018) 012102 doi:10.1088/1757-899X/434/1/012102

Effect of different extender on the quality of sexing sperm before freezing in limousin cattle

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Abstract. The aim of this study was to determine the quality of sexing sperm before freezing by Percoll density gradient centrifugation method using egg yolks tris aminomethan and andromed® extender. This research method was experimental laboratory studies, the treatment egg yolks tris aminomethan and andromed® extender, parameters observed in the study include motility, percentage of viability and abnormalities fresh sperm before freezing. Sexing results were analyzed using completely randomized design, each treatment consisted of 10 replications. The results showed that both sperm motility and abnormalities using andromed had significant ($P < 0.05$) than using egg yolk tris aminomethan. Glicerol in andromed as a cryoprotectant in the groove mechanism of the reaction cell preservation were to a decrease in freezing cryoprotectant medium, to the protect cell membranes, to curb the influence of increasing concentration, as well as to change the shape and size of ice crystals. It might be concluded that the quality of spermatozoa using andromed better than using egg yolk tris aminomethan.

1. Introduction

Artificial insemination technology could be enhanced [1] in value by using the program which had generated calf sex as expected, because of very supportive breeding programs in the selection of breeds. The advantages of separation of sperm were capable of supporting efficiency in dairy and beef cattle, because cows could give birth with the expected sexed to the purpose of these farms [2, 3]. Percoll density gradient centrifugation was used as a way to separate the X and Y sperm. This was because the percoll was a medium that could be created with various density which did not penetrate the cell membrane and had a low vikosity [4]. The method of separation by percoll density gradient centrifugation had the opportunity to develop. This was evidenced by the ability of sperm to fertilization with the ovum, showing the sex of the cattle in accordance with expectations [5].

Centrifugation and freezing process could cause stress to the cells that often-produced damaged sperm membrane and decreased sperm motility and viability [6, 7]. To be able to maintain the quality

of the sperm in order to stay well after centrifugation, during cooling and freezing of the extender was required so that spermatozoa did not have proper cold shock. Yolk sperm extender as a cryoprotectant was at risk of unhygienic and difficult standardized. Although the sperm without egg yolk extender was readily available but the extender with the yolk still widely used for freezing sperm [8].

2. Method

The research was conducted in the laboratory of Artificial Insemination Center (BBIB) Singosari and Universitas Kanjuruhan Malang. Sperm samples used were obtained from the fresh semen of Limousin cattle that are kept in BBIB Singosari. The criteria of semen used were ≥ 2+ mass motility, individual motility ≥ 70%. The shelter of the semen was twice a week for each individual.

Parameters observed in this study were the sperm motility, the percentage of sperm viability, and sperm abnormalities of fresh semen before freezing. Semen after sexing data was analyzed using completely randomized design. Each treatment consisted of 10 replications. Based on data analysis obtained, if there were significant differeces, then the futher test using Duncan's Multiple range test were conducted.

3. Result and discussion

The research was conducted in the laboratory of Artificial Insemination Center (BBIB) Singosari and Universitas Kanjuruhan Malang. Sperm samples used were obtained from the fresh semen of Limousin cattle that are kept in BBIB Singosari. The criteria of semen used were ≥ 2+ mass motility, individual motility ≥ 70%. The shelter of the semen was twice a week for each individual.

The quality of sperm examination on the study includes the volume, colour, concentration, mass motility, individual motility, percentage viability, sperm abnormalities, and total motile sperm.

Table 1. The quality of sperm used in research.

Examination	Average (Mean±SD)
Volume	7.20 ± 1.29
Colour	White
Ph	6.4 ± 0
Mass Motility	++
Individual Motility (%)	700
Concentration (bilion/ml)	1352.20 ± 173.90
Viability	86.81 ± 5.75
Abnormality	5.95 ± 2.01

The quality of sperm on research showed that the sperm used was appropriate for further processing. The percentage of sperm motility obtained from Limousin cattle microscopic examination was 70 ± 0% with a concentration of 1352.20 ± 173.90 million sperm/ml. The percentage of motility and concentration of sperm met the requirements for further processing, because a minimum percentage of motility and concentration produced should be 70% and not less than 500 million sperm/ml [9]. Sperm used must have a percentage of motility over 50% with a concentration of more than 500 million sperm/ml [10]. Percentage of motility of fresh sperm in this study was high. It was intended to use more sperm which was able to survive during the separation process.

According to [11] that the sperm which had motility percentages above 70% better survival than if lower than 70%. Examination of the concentration needed to be done, because according to [12] that the concentration of spermatozoa could be used to predict fertility of bulls. The percentage of semen abnormality of 5.95±2.01% indicated the use of sperm was eligible for further proceedings according to [13] sperm abnormality should not exceed 20%. The quality of sperm was used in this study was the semen that had good quality. It was intended that the sperms were better to be able to survive during the process of separation by Percoll density gradient centrifugation method.

3.1. Sperm quality before freezing

3.1.1. Percentage of sperm motility. The percentage sperm motility before freezing was shown in table 2.

Table 2. The percentage sperm motility (%) before freezing.

Layer	Treatment	
	Egg Yolk Tris Aminomethan	Andromed®
Top	32.50 ± 16.2[a]	40.00 ± 12.69[b]
Bottom	51.00 ± 3.16[a]	53.00 ± 7.53[b]

Table 2 showed that the percentage of motility before freezing using andromed extender was better to use egg yolk tris. The results of statistical tests showed that there was a profound difference ($P < 0.01$) on the percentage motility of spermatozoa before freezing by using egg yolk tris Aminomethan and Andromed extender. These extender provided a very significant difference ($P < 0.01$) on the percentage motility of sperm before freezing at the top and bottom layers with the extender andromed given resulting of sperm motility before freezing was higher than among egg yolk tris Aminomethan.

The separation of sperm by centrifugation method resulted in damage to membrane structure, because the damage to the membrane of sperm were more susceptible to cold shock in the cooling process. This was supported by [4] that the separation of sperm was more susceptible to cooling than sperm without separation. This was because at the time of separation of sperm had damaged tail or head, so that the membrane structure of sperm was partially damaged and disrupted its function.

Further [2] stated that the roles of glycerol as a cryoprotectant in the groove mechanism of the reaction cell preservation were to a decrease in freezing cryoprotectant medium, to the protect cell membranes, to curb the influence of increasing concentration, as well as to change the shape and size of ice crystals. Glycerol with the extender should be incorporated into the semen that had been mixed with a extender without glycerol after the temperature reaches 5oC refrigerated no more than 2 hours.

3.1.2. Percentage of sperm viability. The percentage of sperm viability before freezing was shown in table 3.

Table 3. The percentage sperm viability (%) before freezing.

Layer	Treatment	
	Egg Yolk Tris Aminomethan	Andromed®
Top	74.99 ± 8.26	76.25 ± 10.50
Bottom	77.74 ± 9.91	79.88 ± 10.51

Table 3 showed viable sperm was safe for further proceedings, because the standard for the percentage of freezing process of living was more than 60%. The results of statistical analysis showed that the addition of egg yok tris aminomethan and andromed extender in the freezing process had no significant difference ($P > 0.05$) on the percentage of spermatozoa viability before freezing the top layer as well as on the bottom layer.

Glycerol could cause a decrease in the percentage of live sperm during cooling. This was caused by chemical poisoning in the sperm cells that caused damage to the plasma membrane, so that negrosin eosin staining of the cells could absorb the color. According to [14] that the addition of glycerol would cause excessive osmotic pressure and dehydration of colloidal suspensions both intra-and extracellular concentration of the solution to be toxic and lethal.

The decrease of the percentage of live sperm could be caused by the process of equilibration and freezing, during the process in the sperm formed ice crystals intaseluler, also occurs chemically poisoned by glycerol and cold shock effect due to changes in temperature, it was supported by [15] who argue that storage in isotonic equilibration became membrane damage. Therefore, glycerol was added gradually to give protection against dehydration, high toxic effects of glycerol. While at the beginning already contained andromed of glycerol.

To reduce damage to sperm during the cooling process was a necessary addition of a substance as the surrounding environment that could protect from the effects of cold shock and lechitin lipoproteins contained in egg yolk and milk. Egg yolk on the physical and biological properties of chemical cryoprotectant with semi-permeable cell membrane, extracellular cryoprotectant with relatively large molecules can not penetrate the cell membrane. Egg yolks contain amino acids that are the source of protein and ascorbic acid composition of lipoproteins that serve to protect the sperm plasma membrane during the cooling process [16].

3.1.3. The percentage of sperm abnormalities. The percentage of abnormal sperm before freezing was shown in table 4.

Table 4. Abnormalities of sperm before freezing Percentage (%).

Layer	Treatment	
	Egg Yolk Tris Aminomethan	Andromed®
Top	13.87 ± 5.12^{b}	9.67 ± 4.76^{a}
Bottom	9.63 ± 2.64^{b}	8.75 ± 3.54^{a}

The results of statistical tests showed that there was a profound difference ($P < 0.01$) on sperm abnormalities before freezing on the top layer in the group. The addition of egg yolk tris aminomethan and Andromed extender before freezing processes provide a very significant difference ($P < 0.01$) on sperm abnormalities before freezing on the top layer, where the addition of extender andromed provide abnormalities of sperm before freezing on the top layer was not different from egg yolk tris aminomethan extender, as well as at the freezing process. Abnormalities of the lower layer shows a lower value than the top layer and seen that the abnormalities of sperm using the andromed better than egg yolk tris aminomethan extender. Abnormalities of sperm was caused due to the influence of centrifugation which resulted in damage to membrane structure occurs due to collisions between sperm with medium and the tube wall by centrifugal force. Separation by centrifugation method resulted in damage to the structure of the sperm membrane [2].

The factors that cause an increase in abnormalities during cooling and freezing processes include extender, change in temperature (refrigeration and freezing), the speed of cooling and process of preparations [17]. Increased abnormalities during the extender process was a change in pressure due to the expenditure osmose ions, causing severe dehydration that will damage the cells shrink and the cell membrane.

Abnormalities before freezing sperm of separation with Percoll density gradient centrifugation was still below the AI standard 20% so it's worth it for the AI. It showed the yolk contained in the extender containing egg yolk tris aminomethan lechitin and lipoproteins serve to protect and preserve the integrity of the cell envelope lipoproteins and prevent cold shock. Cold temperature storage could enable glycerol to keep it functioning as a protection and could interact with the cell membrane so that the cell membrane becomes pliable, not brittle and not easily broken [18].

4. Conclusion

Before freezing sexing sperm quality using andromed better than using egg yolk tris aminomethan.

Acknowledgements
We acknowledged Ristekdikti grand: PEKERTI. We thank to LPPM Universitas Kanjuruhan Malang.

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