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Optimization of Bovine Sperm Sexing: Modification of Column Length and Separation Time

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ABSTRACT: The aim of this research is to determine the effect of sperm separation using albumin column with the modification of the column length and time of separation on sperm quality and the effectiveness of sperm separation. Semen was collected using an AV, evaluated and then separated using albumin column technique. The design of this research was factorial 2x2 with two treatments of length column: 2 ml (control) vs 3 ml (treatment) and two treatments of time separation (15 vs 30 minutes. The percentages of motility, live and dead and TAU of separated sperm were better in upper fraction compare to the lower fraction in both of the control group and the treatment group. The sperm motility in control group was 74.0 - 75.6% and 62.5 - 63.9% for upper fraction and lower fraction respectively. Compare to the treatment group were 70-70% dan 46 – 50% % for upper fraction and lower fraction respectively. Sperm concentration had a tendency to be reduced in the lower fraction and treatment group. The results form the control group showed that the ratio of X and Y sperm could be changed from 48%:52% (fresh semen) to 57%: 42.5% (separation time: 15 minutes) and 83.75%:16.25% (separation time: 30 minutes) for the upper fraction. For the lower fraction the ratio of X:Y were 30%:70% (15 minutes) and 26.25%:73.75% (30 minutes). In the other hand, form the treatment group of 30-minute time separation, showed that the ratio of X and Y were 85%:15% (upper fraction) and 21.70%:78.30% (lower fraction). From the results, it can be concluded that the technology of sperm separation column albumin is still effective to separate X and Y sperm both in sperm quality after separation and the sperm X and Y ratio achieved.

Keywords: Bovine, Sexing, Sperm, Albumin column

INTRODUCTION

Effort to increase the efficiency of using Artificial Insemination (AI) in cattle is how to obtain the effectiveness of sperm separation (sexing sperm) technology to separate X and Y sperm chromosomes. Sexing sperm technology can improve livestock production if farmers can get calves sex as desired. Many experiments have been done to control the sex ratio of calves from cows conception. Ericsson and Glass (1982) reported the X and Y sperm separation media using Bovine Serum Albumin (BSA) in human, based on motility differences. X and Y sperm separation by using egg-albumin media has also been reported by some researchers (Saili, 1999). To improve semen quality post-separation using egg-albumin has been reported with adding of isomethyl-xhantine (Sianturi *et al.*, 2003) and cholesterol, gluthation (Sianturi *et al.*, 2007). From many methods of sexing sperm, the most common method is based on the difference in density or motility, but there are still not as expected, where the results of pregnancy rates and the expected calves-sex are still unsatisfactory. Sexing sperm technology with high percentage of sex-matched is by Flow Cytometry, but these tools are very expensive and the concentration of sperm produced is very low, and there are concerns of the damage and gene mutations as a result using laser treatment (Seidel *et al.*, 1997). The purpose of this study, is to examine the effect of modification egg-albumin column/gradient on the effectiveness of sperm sexing in dairy cows.

MATERIALS AND METHODS

Two dairy bulls used as a source of semen. Semen collected using an artificial vagina (AV) two times a week and only good quality semen used for the research. The design of this research was factorial 2x2 with two treatments of length column: 2 ml (control) vs 3 ml (treatment) and two treatments of time separation (15 vs 30 minutes).

Factor 1, Modification of column length

K : Control, 2ml Tris citrat buffer + 30% v/v egg albumin (lower fraction) and 2 ml Tris citrat buffer + 10% v/v egg albumin (upper fraction).

P : Treatment, 3ml Tris citrat buffer + 30% v/v egg albumin (lower fraction) and 3 ml Tris citrat buffer + 10% v/v egg albumin (upper fraction).

Factor 2, Time of separation

T1 : separation time 15 minute

T2 : separation time 30 minute

Semen to be separated diluted two times with diluent appropriate to treatment media, and 1 ml placed on the surface layer of albumin (separation media), then allowed for 15 and 30 minutes for sperm separation process. And took 2 ml from each of the upper and lower fractions, diluted with diluent and centrifuge for 10 minutes at a speed of 2500 rpm. Sediment obtained were diluted with diluent Tris-citrate + 20% v/v egg-yolk, then evaluated and freezed with routinely freezing method of Balitnak Laboratory of Reproduction.

The parameters observed, percentage of X and Y sperm based on morphometry of sperm wide head, sperm concentration, percentages of motility, live and dead sperm, intact apical ridge (IAR) before and after sperm separation and after frozen of the sexed semen. All data were analyzed statistically followed Steel and Torrie (1993).

RESULT AND DISCUSSION

Quality of fresh semen obtained generally meet the criteria of a good bull semen. Fresh semen, had 1-15 ml volume, creamy white color, viscous consistency, mass movement (+++) and had an average of live sperm lives more than 80% (Toelihere, 1985). The morphometry observation of the fresh semen, showed that the percentage of spermatozoa X and Y approaching 50%: 50% (X: Y: 48%: 52%), this is in accordance with the general state of the composition of spermatozoa X and Y in the fresh semen is 50%: 50%, and after fertilization, the zygote will be 50% male and 50% female (Mc.Donald, 1989).

In Table 1., it shows the percentage sperm motility of separated (sexed) semen from control and treatment group. The result shows that generally better sperm motility in the control group compare to the treatment group, that are: 74.0 to 75.6% and from 62.5 to 63.9% for the upper and lower fractions, respectively. Compared to the treatment column 70-70% and 46-50% for for the upper and lower fractions, respectively. In the treatment group, in lower fraction, sperm motility is fairly low, 46% - 50%, where with this low motility are not good to be frozen. It maybe because the sperm trying to pierce the longer of fraction column, that was 3 ml of sexing media (equivalent to approximately 3-cm height column) and more concentrated with high viscosity solution. Surely, it will drain energy and motility of sperm will be greatly decreased because the sperm have to penetrate a longer column of separation media consisting of two layers/columns with a high viscosity of 10% and 30% egg albumin.

Tabel 1. The effect of modified length albumin column and time of seperation on the percentage of sperm motility (%M)

Column Treatment	Separation Time	Upper Fraction	Lower Fraction
Control (2 ml)	15 minutes	74.0 + 5.5	62.5 + 2.7
	30 minutes	75.6 + 5.3	63.9 + 7.0
Treatment (3 ml)	15 minutes	70.0 + 10.0	46.0 + 8.9
	30 minutes	70.0 + 7.1	50.0 + 13.1

Equivalent to sperm motility, the percentage of live sperm (% LD) in the lower fraction of the treatment group also decreased by 63.4% and 77.4% for the time of the separation of 15 minutes and 30 minutes respectively (Table 2.). As for the upper fraction of the treatment group, % LD still about 83-84%. From the results of Tables 1 and 2, it can be said that although sperm motility has also declined, but the percentage of live sperm were still higher.

Tabel 2. The effect of modified length albumin column and time of seperation on the percentage live-sperm (%LD)

Column Treatment	Separation Time	Upper Fraction	Lower Fraction
Control (2 ml)	15 minutes	86.6 + 7.1	81.2 + 4.1
	30 minutes	85.6 + 4.9	80.9 + 5.5
Treatment (3 ml)	15 minutes	83.0 + 6.4	63.4 + 13.9
	30 minutes	84.3 + 5.6	77.4 + 7,3

Table 3. shows the percentage of sperm that have intact apical ridgre (% IAR). From the table it appears that in general % IAR are still good, ranging from 78-89% and deserves to be frozen. IAR is tendency lower with a longer separation time (30 minutes) for both control and treatment groups.

Tabel 3. The effect of modified length albumin column and time of seperation on the percentage of Intact Apical Ridge (%IAR)

Column Treatment	Separation Time	Upper Fraction	Lower Fraction
Control (2 ml)	15 minutes	87.2 + 2.6	83.8 + 4.7
	30 minutes	77.7 + 13.2	79.4 + 10.4
Treatment (3 ml)	15 minutes	89.0 + 8.7	85.8 + 5.6
	30 minutes	80.3 + 8.5	79.6 + 11.6

This is certainly acceptable, with a longer time, the majority of spermatozoa trying to penetrate more viscous liquids media damaged the sperm acrosome/apical.

Table 4 describes the effect of column modification and duration time of separation to the concentration of spermatozoa. The sperm concentration in the control group and upper fraction were 186.3 and 198.6 million spermatozoa/ml for separation time 15 and 30 minutes respectively. For the bottom fraction were 23.8 and 92.5 million spermatozoa/ml for 15 and 30 minutes. As for the middle fraction (not in Table), the concentration of spermatozoa is still quite high at 187.5 and 155.0 million spermatozoa / ml.

Tabel 4. The effect of modified length albumin column and time of separation on the sperm concentration

Column Treatment	Separation Time	Upper Fraction (..x 106 sperm/ml)	Lower Fraction (..x 106 sperm/ml)
Control (2 ml)	15 minutes	186.3 + 44.2	23.8 + 6.3
	30 minutes	198.6 + 76.9	92.5 + 50.1
Treatment (3 ml)	15 minutes	136.0 + 32.7	12.5 + 2.9
	30 minutes	177.0 + 49.7	87.0 + 23.9

From these results, the concentration of sperm yields for lower fractions was very low, 23.8 million sperm/ml for 15 minutes. This concentration is too low and would be difficult to further processing, to be frozen or to be chilled (chilled semen). The smaller sperm concentration obtained, is likely the greater Y sperm purity filtered.

The data in Table 5, albumin column separation techniques can change the ratio of X: Y of 48: 52% (fresh semen) to 57.5: 42.5% (the separation time 15 minutes) and 83.75: 16.25% (the separation time 30 minutes) for upper fraction and control group. From this comparison, 15 minutes of separation time was not enough to separate the X and Y sperm, but after 30 minutes, it was a tendency to increase the percentage of X sperm. In contrast, for the lower fraction of control group, changed the ratio of X and Y to 30: 70% (15 min) and 26.25: 73.75% (30 min).

Tabel 5. The effect of modified length albumin column and time of separation on the ratio of X and Y sperm (%)

Perlakuan kolom	Upper Fraction		Lower Fraction	
	X-sperm	Y-sperm	X-sperm	Y-sperm
Control (2 ml)				
15 minutes	57.50	42.50	30.00	70.00
30 minutes	83.75	16.25	26.25	73.75
Treatment (3 ml)				
15 minutes	62.50	37.50	42.50	57.50
30 minutes	85.00	15.00	21.70	78.30

Whereas in the treatment group, for 15-minutes separation time, obtained X and Y proportion of 62.50: 37.5% (upper fraction) and 42.5: 57.50% (lower fraction). For 30-minutes, the ratio of X and Y percentage were 85: 15% for upper fraction and 21.70: 78.30% for the lower fraction.

In general, the results showed that the 15-min separation time is not enough to obtain optimal results, especially in the upper fraction, because it is still a small portion of Y sperm that swim down to the lower fraction. After the 30 minutes of time separation, then the results of the X-sperm percentage ratio has further improved, ie. 83.75% and 85% respectively for the control and treatment groups, because most of Y- sperm already swim down through the lower-fractions. From all of research results, sexing sperm technology using egg albumin columns is still effective to separate X and Y sperm, in terms of quality and ratio of X and Y sperm obtained. The sexed sperm concentration in this study is still inadequate to be processed further or to be frozen. This is possibility caused by several factors, beside as sexing sperm techniques, separation media and separation time which is still not optimum and of course, the quality of fresh semen also affect the results of this study.

CONCLUSION

1. The time separation of 15-minutes is not adequate to get the ratio of X and Y sperm as expected.
2. The separation of sperm using albumin column, in the control group may alter the ratio of X: Y of 48: 52% (fresh semen) to 83.75: 16.25% for the upper fraction and 30-minutes of separation time, while for the lower fraction to 26, 25: 73.75%.
3. In the treatment group, 30-minutes of time separation, the gain of percentage ratio of X and Y sperm was 85:15% for upper fraction and 22:78% for lower fraction.
4. Sexing sperm technology using egg native-chicken albumin is still effective to separate X and Y dairy bull sperm, both in sperm quality after separation and the sperm X and Y ratio achieved, but sexed sperm concentration produced from lower fraction is still inadequate.

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