Evaluation the Natural Proportion of X-Y Chromosome Bearing Sperm of West Java Local Ram Using Morfometric Methode

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ABSTRACT

Local Ram has a great contribution to supply of meat consumtion especially in West Java. Reproductive biotechnology was used to ensure meat production such as sperm sexing technology. As a preliminary study of Sperm Sexing technology in Local ram, we should know the natural ratio of X-Y chromosome bearing sperm to develop the sperm sexing methode. The objective of this study were to evaluate the natural ratio of X-Y chromosome bearing sperm in Local Ram. The object of this research was semen from 30 Local Ram, and then was made differential preparat for counting the size of head sperm as much 200 sperm cell. Sperm whose head size is greater than the average is categorized as X chromosome bearing sperm (X sperm), and smaller than average is categorized as the Y chromosome bearing sperm (Y sperm). Analisys data consist of percentage of X and Y chromosome bearing and then will avearage from 30 local Ram. The result of this study showed that of 30 Local Ram that were sampled had a sperm head length between 8.14 to 9.31 μm with an average of 8.66 μm; Sperm head width between 4.50 to 5.39 μm with an average of 4.93 μm; Sperm head area ranges from 22.97 - 59.30 μm^2 with an average of 36.82 μm^2. Based on the size of the sperm head, obtained the average proportion of sperm X and Y of 50.70% (X) and 49.30% (Y). Based on the research data, it is concluded that the proportion of X and Y chromosomes bearing sperm of West Java Local Ram approached the proportion of 50: 50, indicating that the birth of male and female lamb in Local Ram had equal opportunity value.

Keywords: X-Y Chromosome bearing sperm, Local Ram

INTRODUCTION

Local ram have a major role in the provision of meat to fullfill the needs of protein consumtion, especially in West Java. In an effort to improve the productivity of Local Ram, reproductive biotechnology is one of metode can be used to achieve that goal. A series of studies have been conducted to obtain some information about the characteristics of Local Ram reproduction as reported by Solihati *et al*. (2016a) that the most optimal age to produce the best semen quality that is at the age of 2-3 years. In addition, it has been reported that the quality of Local Ram semen qualify to be used for the artificial insemination (AI) programe either in the form of fresh, chilled semen or frozen semen, where the level of 5% glycerol produces the best post-thawed semen quality include motility, intact plasma membrane, and abnormality of West Java local Ram (Solihati *et al*, 2016b) and 5% glycerol levels resulted in the best intact acrosom sperm (Solihati *et al*, 2016c). Efforts to improve the quality of local sheep semen have also been done by adding antioxidants such as α-tocopherol and glutathione, which concluded that glutathione is the best antioxidant for viability in ram semen at puberty (Solihati *et al*, 2015).

These efforts to improve the quality of Local Ram semen are still being undertaken to improve more effective outcomes in achieving the goal of increasing the rams population for meat consumption fulfillment. Biotechnology sexing sperm will be attempted as an approach to obtain a higher proportion of male births. One of the sperm sexing techniques is by colum albumen method based on the principle of difference in size between X chromosome bearing sperm (sperm X) and Y chromosome bearing sperm (Y sperm), and is known that X sperm has bigger size than Y sperm. The difference can be made a benchmark to knowing the proportion of X and Y sperm. So far information on the natural ratio of X and Y sperm of the Local Ram has not been reported, which can be used as a reference to determine the success of the sexing method. This study aims to determine the natural ratio of X-Y sperm to Local Ram as a reference to know the success of sperm sexing method in Local Sheep.

MATERIALS AND METHODS

The object of this research was semen from 30 Local Ram, more than 1 years in old. Semen was collected with an artificial vagina (AV) and then was made differential preparat for counting the size of head sperm. Every Local Ram semen was made one preparat used eosin 2% solution. Every preparat was counted for 200 sperm cell.

Determination of X-Y chromosome bearing sperm based on morfometric evaluation use aplication DP2-BSW with magnification 10x100. Morphometry is a method of estimating sperm X and Y by knowing the wide of sperm head (μm^2). The width of the sperm head is calculated by the integral method of Riemann (Purcell and Varberg, 1987) and regression analysis method (Steel and Torie 1995) to determine the relationship between the length and width of the sperm head. Obtained values of each size which will then be compared with the average size of each test of 200 spermatozoa from each Local Ram sperm. Sperm whose head size is greater than the average is categorized as X chromosome bearing sperm (X sperm), and smaller than average is categorized as the Y chromosome bearing sperm (Y sperm).

The calculation formula for the spermatozoa head was as follows:

$$SHA = (0{,}8988 \times L \times W) - 1{,}63$$

Explanation :

SHA : Sperm Head Area
0.8988 : The correction factor of the integral data to determine the sperm head area of each unit of measure and the regression method to determine the relation of length and width to the size of the sperm head.
L : Length of sperm head
W : Width of sperm head
1.63 : Regression constanta value

Calculation result of sperm head wide of each sperm cell will then be compared with the average of sperm head wide from 200 sperm cell to obtain the percentage of X sperm and Y sperm. Analisys data consist of a percentage of X and Y sperm in each Local Ram and then will avearage from 30 local Ram.

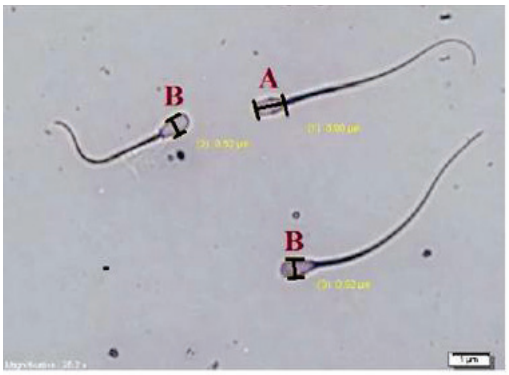

Figure 1. Morfometric evaluation for length, width and wide of sperm head

RESULTS AND DISCUSSION

Size of sperm head and proportion of X-Y chromosome bearing sperm

Determination of X and Y sperm in this study based on the measurement of length, width (figure 1) and wide of the sperm head. Sperm with a larger head area than the average is categorized as sperm X and sperm smaller than the average is categorized as sperm Y. The determination of X and Y sperm refers to some previous research report which states that the wide of the sperm head greater than average were identified as sperm X while smaller sperm head wide was identified as sperm Y (Susilawati, 2014). This is based on the discovery that the X sperm head contains more DNA than the Y sperm, as reported by Mohri *et al*. (1986), that the weight and size of the head of spermatozoa X is caused by the presence of DNA content in the spermatozoa head of X as much as 3-4% larger than the DNA contained in the head of the Y.sperm. Similarly, Sumner and Robinson (1976) reported that the sperm head DNA content is related to the weight of the head mass. In mammals the X chromosome is larger than the Y chromosome and hence contains more DNA. The degree of difference depends upon species and amounts to approximately 2.9% in human spermatozoa, whereas Johnson (1992) reported the degree of difference approximately 3.8% in bulls and as much as about 7.5% in chinchilla. Goodall & Roberts (1976) reported that Y sperm with smaller shapes and sizes, and containing fewer DNAs had higher motility than X sperm.

In this study, the result of morfometric evaluation showed that Local Ram had a sperm head length between 8.14 to 9.31 µm with an average of 8.66 µm; Sperm head width between 4.50 to 5.39 µm with an average of 4.93 µm; Sperm head wide ranges from 22.97-59.30 μm^2 with an average of 36.82 μm^2.

The measurements of morphometric evaluation on Local Ram sperm were greater than those reported by Achdiat (2012) who also studied Local Ram with the result of length 7,30 ± 0,23 µm, width 4,25 ± 0,13 µm and wide 31.01 ± 2.00 μm^2. The results of this study were also larger than those reported by Rizal *et al*. (2003) in Garut sheep, namely the average length of sperm head 6.59 µm (6 - 7 µm), and width of 3.99 µm (3.5 - 4.5 µm). Similarly, reported by Takdir *et al*. (2017) in Garut Sheep with the average result of sperm head length of 6.59±0.14, width of sperm head 4.01±0.01 and sperm head wide 22.01±0.67.

Proportion of X-Y sperm

The resul showed that based on area sperm head, obtained the average proportion of X and Ysperm is 50.70% (X) and 49.30% (Y). This result in accordance with the report from many other animal breeds but not with human and rat sperm.

Bintara (2011) reported proportion of X:Y sperm in Etawah Crossbreed goat is 50.6±1,8 (X): 49.4±1.8 (Y) and in Kacang goat is 49.7±1.7(X): 50.3±1.7 (Y), showed that the proportian of X:Y sperm in two different breed is 50:50. Also Garner *et al*. (1983) reported in

many breed bull and result showed that the X-Y proportion is 49.5 to 50.5% for all breeds. The X-Y peak differences did not vary within each breed, but were significantly different when the breeds were compared.

Table 1. Calculation result of length, width and sperm head wide and proportion of X-Y sperm of Local Ram

No of Ram	Average of length and width sperm head from 200 sperm cell		Average of sperm head wide from 200 sperm cell			Proportion (%)	
	Length	Width	Average	Minimum	Maximum	X	Y
1	8,23	4,65	32,78	25,04	43,86	48,50	51,50
2	8,30	4,70	33,49	26,11	46,84	46,50	53,50
3	9,29	5,39	43,49	29,20	51,59	64,00	36,00
4	9,32	5,20	42,02	26,40	53,85	56,50	43,50
5	8,98	5,35	41,73	27,39	59,30	54,50	45,50
6	8,79	5,09	38,58	30,38	49,44	50,00	50,00
7	8,20	4,50	31,59	22,97	40,75	51,50	48,50
8	8,65	4,71	35,07	25,95	45,40	47,50	52,50
9	8,75	4,85	36,53	29,59	45,03	48,00	52,00
10	8,48	4,80	35,00	28,30	41,61	54,00	46,00
11	8,42	4,78	34,58	26,14	41,75	51,00	49,00
12	8,45	4,77	34,61	26,28	43,10	52,50	47,50
13	8,48	4,84	35,29	26,75	46,42	50,00	50,00
14	8,53	4,87	35,70	28,75	43,45	51,00	49,00
15	8,79	5,26	40,02	28,21	58,89	41,00	59,00
16	8,56	5,04	37,19	29,57	55,26	50,00	50,00
17	8,95	5,12	39,58	33,30	48,30	48,00	52,00
18	8,40	4,86	35,08	29,02	42,16	52,00	48,00
19	8,14	4,63	32,25	23,28	40,78	54,50	45,50
20	8,62	5,01	37,18	29,07	46,26	49,00	51,00
21	8,56	4,83	35,52	25,26	45,11	52,50	47,50
22	8,45	4,94	35,92	26,80	48,92	47,00	53,00
23	8,77	5,03	38,04	31,21	52,92	45,50	54,50
24	9,08	5,07	39,77	30,28	53,06	48,50	51,50
25	8,51	4,98	36,51	27,17	48,36	49,50	50,50
26	8,49	4,68	34,16	25,91	42,24	52,00	48,00
27	8,85	5,09	38,84	31,71	49,14	50,00	50,00
28	8,61	5,03	37,32	27,98	47,62	51,50	48,50
29	8,96	4,95	38,24	30,77	48,78	50,50	49,50
30	9,06	4,91	38,46	27,79	50,53	54,00	46,00
	8,66	4,93	36,82	27,89	47,69	50,70	49,30

In human, Chaudhary *et al*. (2014) reported that they have found more (52%) X than Y (48%) bearing sperms. In mouse (epididymal sperms) also they observed a preponderance of X (55.5%) as compared to Y (44.5%) bearing sperms. A probable reason for this could be the presence of meiotic drive elements which could lead to incapacitation or fragmentation of Y bearing sperm or preferential elimination of Y bearing sperm through apoptosis mechanism. This is also supported by the evidence of more aneuploidy with Y-bearing

spermatozoa (~1.5X more with Y bearing sperms; 192 aneuploid Y sperms and 124 aneuploid X sperms).

CONCLUSIONS

Based on the research data, it is concluded that the proportion of X and Y chromosomes bearing sperm of West Java Local Ram approached the proportion of 50 : 50, indicating that the birth of male and female lamb in Local Ram had equal opportunity value.

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