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Profile of Protein Tyrosine Kinase in Seminal Plasma of Merino Sheep using Technique of Sodium Dodecyl Sulphate Polyacrilamide Gel Electrophoresis

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Abstract : The research aims to find out profile of protein tyrosine kinase in seminal plasma of Merino sheep.The semen was obtained from two Merino sheep collected by using artificial vagina. Next, separation of spermatozoa from seminal plasma was carried out using centrifugation with the speed of 4000 rpm for 40 minutes. Seminal plasma obtained was purified using centrifugation with the speed of 3000 rpm for 10 minutes, then media of PBST-PMSF were added as much as 5 times of seminal plasma volume and after that, vortex was done to mix them homogeneously . Then, sonication for 10 minutes and centrifugation with the speed of 6000 rpm for 10 minutes were carried out on them. Supernatant obtained was added with etanol with the ratio of 1:1. Finally, analysis of protein tyrosine kinase tapes was conducted using the technique of SDS-PAGE. The research showed that there were 13 protein tapes in each sample of seminal plasma, that is, 49.63 kDa, 139.7 kDa, 114.97 kDa, 109.3 kDa, 97.33 kDa, 93.83 kDa, 86.23 kDa, 77.6 kDa, 64.6 kDa, 52.3 kDa, 41.93 kDa, 38.13 kDa, dan 34.5 kDa. The research concluded that protein tyrosine kinase in seminal plasma of Merino sheep was on the 6th tape with molecular weight of 93.83 kDa.

Key words : protein tyrosine kinase, SDS-PAGE, Merino sheep.

Introduction

Mutton like chicken, can be accepted by all levels of society (Sudarmono and Sugeng, 2003). It is known from higher demand of mutton which increases 2.7% per year averagely, however, it is not followed by the availability of sheep breed in the country (Mulyono dan Sarwono, 2014).The effort to increase reproduction productivity can be reached by one of them is biotechnology . Reproductive biotechnology which is acceptable to breeders to increase cattle production is superovulation, that is, reproduction which is used to yield multiple births and technology of artificial insemination (AI) which aims to utilize potential of a superior male maximally. Artificial insemination done to sheep has not developed yet and is still experimental. It is caused by the management of sheep breed in Indonesia which is still done traditionally (Hardijanto dkk., 2010).

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Semen usually used at AI program is frozen semen. It is intended to broaden the scope of semen distribution, and to lengthen storage span of the semen. However, in some cases, freezing and thawing can induce damage of spermatozoa which leads to decreased quality such as decrease of motility as much as 40% and decrease of viability as much as 20-30%, decrease of plasma membrane integrity, increase of chromatin condensation errors, and increase of DNA fragmentation. Decreased quality of spermatozoa will lead to decreased ability of spermatozoa to fertilize ovum (Inounu, 2014). In addition, low fertility rate ofsheep can be induced as sheep semen is more sensitive to cold shock compared to other species (Muino-Blanco *et al*., 2008).

Due to the problem at making frozen semen above, at the moment there are many researches using supplementation medium which is added with diluting medium which can minimize damages of spermatozoa due to freezing process. One of the supplementation media which can be used as frozen semen mixture is tyrosine kinase. Tyrosine kinase is one of protein molecules in plasma membrane of spermatozoa (Morales and Llanos., 1996). Naz dan Ahmed (1994) also stated that several spermatozoa membranen proteins in rats are able to bind ovum zona pellucida proteins with the molecular weights of 95. 63, and 14.18 kDa. Yet, the protein with molecular weight of 95 kDa has the most potential autophosphorylation activity.

Research Methodology

1.Step of Collecting fresh semen of Merino sheep

Semen was obtained from 2 male Merino sheep at animal farm and Frozen Semen Unit of Faculty of Veterinary Medicine Airlangga University in Gresik. Female Merino sheep was used as stimulator then semen released was collected in artificial vagina which was given warm water and rubbed with Vaseline.

2. Step of Separating Spermatozoa from Seminal Plasma of Merino Sheep

Macroscopic and microscopic examination was conducted on the semen obtained.Next, the semen was moved to centrifuge tube for centrifugation with the speed of 4000 rpm for 40 miutes to separate pellet (spermatozoa) from supernatan (semnal plasma). Then, it would be the upper part and the lower one, the upper part was seminal plasma which was taken and put into microtub and kept in the freezer (Susilowati dkk.,2010)

3.**Step of Purifying Protein**

Seminal plasma needed to be purified to find out protein in seminal plasma. Purifying process started with centrifugation on seminal plasma for 10 minutes with the speed of 3000 rpm. After that, PBST-PMSF was added five times of the plasma volume and vortex was done to mix them homogeneously. Sonication was done in 10 minutes and another centrifugation was carried out with the speed of 6000 rpm for 10 minutes. After the centrifugation, plasma was separated from sediment. The plasma was added with etanol 1:1 and next it was kept for 1 day and then running sds-page process was conducted (Aulanni'am, 2005)

4.Step of af Analyzing Protein Tape using SDS-PAGE Technique

Steps of SDS-PAGE technique cover the making of separating gel, the making of stacking gel, it is diluted in running on palate, coloring,then soaked in destaining solution for washing. First step was putting separating gel.into electrophosis through the wall until it reached underneath upper limit. When separating gel was equal and hard,put stacking gel through the wall untill it was full, put comb and wait until gel was formed. Then, comb was taken and cleaned from the gel residue with tissue. The gels formed were moved and put into the chamber. then soaked in running buffer, then the gels were put into sample print and running was conducted.

The next step was adding process or injecting sample..As much as 35 µl seminal plasma was put into the hole of the print with tip 200 µl. Then, chamber was conected into Biorad equipment, power supply was turned on at 130 V, 30 mA for 1.5 hours. If gel reaction reached the bottom, it then was turned off and plate was opened and separated. The result was gel formed sheets which were colored coomasie blue and shaken for 30 minutes. After that, it was taken out and added with destaining liquid and shaken again for 30 minutes. When the liquid looked blue, it must be changed again with new destaining liquid until the liquid looked white and it resulted in several protein tapes. Molecular weight would also be seen at SDS-PAGE gel print (Rantam, 2003).

Result and Discussion

Examination of Fresh Semen of Merino Sheep

Macroscopic examination covers examination on volume, level of acidity or pH, consistency, smell, and color. Microscopic examination covers mass movement, individual movement and concentration. Data on macroscopic and microscopic examination can be seen in Table 1 and Table 2.

Table 1. Result on Macroscopic Examination of Merino Sheep Spermatozoa

Collection	Volume	Smell	Color	pH	Consistency
1	1.5 ml	typical	cream	6-7	thick
2	1.5 ml	typical	cream	6-7	thick
3	1.5 ml	typical	cream	6-7	thick
Average	1.5 ml	typical	cream	6-7	thick

Table 2. Result on microscopic examination spermatozoa of Merino sheep

Collection	Mass movement	Motility (%)	Speed	Concentration million/ml
1	++	85	3	1174
2	+++	85	3	1565
3	+++	85	3	2003
Average	++/+++	85	3	1580

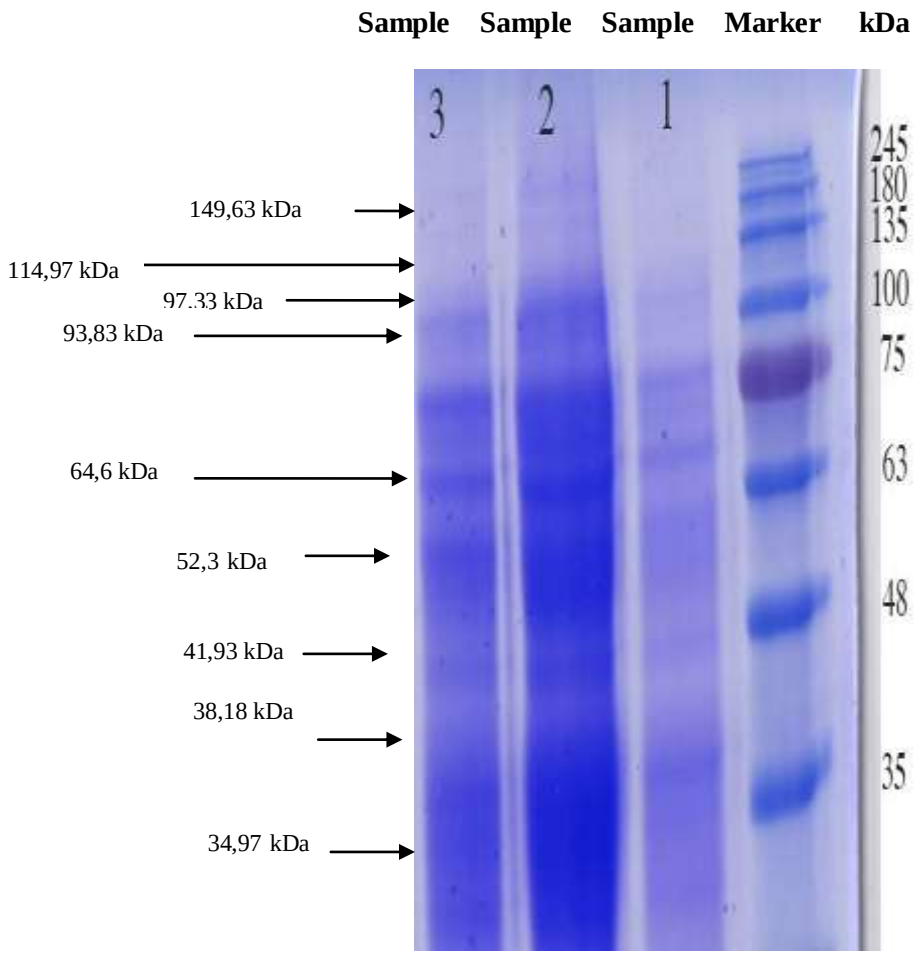

Figure 1. Profile of Protein TyrosineKinase Tapes in Seminal Plasma of Merino Sheep

Result of Protein Tyrosine Kinase Tapes in Seminal Plasma of Merino Sheep

Molecular weight of each protein is determined by equation of regression line based on the value of Rf or Retardation factor.Then, it can be obtained line equation y= -1.532 x + 2.284 which is obtained from standard curve of Rf and log BM marker. Next, from the line equation it can be used to calculate molecular weight of sample using antilog y.

Profile of protein tyrosine kinase in seminal plasma of Merino sheep using SDS-PAGE technique resulted in 13 protein tapes in each sample. It can be seen at figure 1 .

The folowing is data on average molecular weight of each sample obtained.

Table 3. Result on average molecular weights of protein.

No	Sample 1	Sample 2	Sample 3	Average
1	150.5 kDa	150.5 kDa	147.9 kDa	149.63 kDa
2	141.9 kDa	138.6 kDa	138.6 kDa	139.7 kDa
3	116.3 kDa	116.3 kDa	112.3 kDa	114.97 kDa
4	109 kDa	111 kDa	107.1 kDa	109.03 kDa
5	97.5kDa	97 kDa	97.5 kDa	97.33 kDa
6	94.2kDa	93.1 kDa	94.2 kDa	93.83 kDa
7	85.2 kDa	87.8 kDa	85.7 kDa	86.23 kDa
8	76.7kDa	77.6 kDa	78.5 kDa	77.6 kDa
9	63.6 kDa	63.6 kDa	66.6 kDa	64.6 kDa
10	53.3 kDa	51.5 kDa	52.1 kDa	52.3 kDa
11	41.7 kDa	42.4 kDa	41.7 kDa	41.93 kDa
12	37.1 kDa	39.4 kDa	37.9 kDa	38.13 kDa
13	34.7 kDa	34.1 kDa	34.7 kDa	34.5 kDa

From the result above, it can be seen that the sixth protein tape has a.molecular weight of 93.83 kDa which is believed to be the molecular weight of protein tyrosine kinase. It is in line with the statement ofAitken *et al* (1995) which states that protein tyrosine kinase has a molecular weight of 95 kDa.

Discussion

Macroscopic examination on semen quality covers volume, color, smell, pH and consistency while microscopic examination covers mass movement, individual movement and consentration which show that semen is in.good quality and is usable as sample for the next step.

Identification on protein tyrosine kinase in seminal plasma of Merino sheep using technique of SDS-PAGE. SDS-PAGE is a method used to separate molecules based on shape, load and weight in an electrical field (Elrod dan stansfield, 2002). How SDS or Sodium Dodecyl Sulphate works is by surroumding protein molecules, neutralizing all natural loads and destructing secondary and tertiary structures which lead to protein molecules a part based on their size when passing by pores electrophoresis gel (Dorothy, 1993).

Based on analysis on tyrosine kinase identificatlion in seminal plasma of Merino sheep, there were 13 protein tapes in each sample. Molecular weights of each tape averaged 149.63 kDa, 139.7 kDa, 114.97 kDa, 109.3 kDa, 97.33 kDa, 93.83 kDa, 86.23 kDa, 77.6 kDa, 64.6 kDa, 52.3 kDa, 41.93 kDa, 38.13 kDa, dan 34.5 kDa. Particles with the same molecular weights will accumulate at the same spot so that it wil form several different long tapes a part based on molecular weight (Hames, 2004). Success of running SDS-PAGE can be influenced by several factors such as isolate cleanliness, level of isolate purity, protein level in homogenate, and protein mobilization when doing SDS-PAGE analysis (Novagen *et al*., 2007). Pure isolate and good level.of.homogenate protein will. yield.good.and clear protein tapes so that it will facilitate analysis on

molecular weight of protein tapes formed (Kusnoto, 2008). In addition, technical things can influence the result, in the research, holes of the print were not parallel with marker. As a result, protein tapes were not parallel with the markers.

Conclusion

Identification on protein tyrosine kinase in seminal plasma of Merino sheep using technique of SDS PAGE yielded 13 protein tapes in each sample. It had average molecular weights of proteins as follows149.63 kDa, 139,7 kDa, 114.97 kDa, 109.3 kDa, 97.33 kDa, 93.83 kDa, 86.23 kDa, 77.6 kDa, 64.6 kDa, 52.3 kDa, 41.93 kDa, 38.13 kDa, dan 34.5 kDa. Protein tyrosine kinase tape with a molecular weight of 93.83 kDa is believed on the sixth protein tape.

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