

# X-Y Chromosom bearing Sperm Proportion of Local Ram after Sexing with Different Combination of Bovine Serum Albumin (BSA) Concentration

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**Abstract.** The research aims to evaluate proportion of X-Y chromosom bearing sperm after sexing of local ram semen with different combination of BSA concentration. The research object was ten ejaculated semen of local ram, three years old. The research design used CRD (completely randomize design) with four treatments of BSA concentration combination on upper and bottom layer (T1: 3% & 6%, T2: 4% & 6%, T3: 5% & 10%, and T4: 6% & 12%), and 10 repetitions. Data analysis using analysis variance and Duncan's Multiple Range Test. The result showed that combination of BSA concentration was significantly effect on proportion of X-Y chromosome bearing sperm of local ram sperm. The higher average proportion of X- and Y- chromosome bearing sperm was obtained at combination 5% (75.55±1.09% for X) at upper layer and 10% BSA (76.45±1.12% for Y) of bottom layer. The conclusion is that combination of BSA concentration significantly effect on proportion of X-Y chromosome bearing sperm, and concentration of 5% and 10% BSA at upper layer and bottom layer gave the higher proportion of X-Y chromosome bearing sperm of local ram sperm.

**Keywords:** X-Y chromosom bearing sperm, bovine serum albumin, local ram

**Abstrak.** Tujuan penelitian adalah untuk mengevaluasi proporsi kromosom X-Y pada spermatozoa setelah *sexing* pada sperma domba jantan dengan konsentrasi kombinasi BSA berbeda. Objek penelitian adalah 10 sperma yang diejakulasi domba lokal berumur tiga tahun. Rancangan penelitian menggunakan Rancangan Acak Lengkap (RAL) dengan empat perlakuan kombinasi konsentrasi BSA pada lapisan atas dan lapisan bawah (T1: 3% & 6%, T2: 4% & 6%, T3: 5% & 10%, and T4: 6% & 12%), dan 10 ulangan. Analisis data menggunakan analisis variansi dan Uji Jarak Berganda Duncan. Hasil penelitian menunjukkan bahwa kombinasi konsentrasi BSA berpengaruh nyata terhadap proporsi sperma yang mengandung kromosom X-Y dari sperma domba lokal jantan. Proporsi rata-rata sperma yang mengandung kromosom X dan Y yang lebih tinggi diperoleh pada kombinasi 5% (75,55±1,09% untuk X) pada lapisan atas dan 10% BSA (76,45±1,12% untuk Y) pada lapisan bawah. Disimpulkan bahwa kombinasi konsentrasi BSA berpengaruh nyata terhadap proporsi sperma pembawa kromosom XY, dan konsentrasi BSA 5% dan 10% pada lapisan atas dan lapisan bawah memberikan proporsi sperma pembawa kromosom XY yang lebih tinggi pada sperma domba lokal jantan.

**Kata kunci:** Sperma yang mengandung kromosom X-Y, bovine serum albumin, domba lokal jantan

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## Introduction

In the world, in both large- and small-scale farming system sperm sexing has applications. Until now, many of sperm sexing methods have been reported, although still need to be scientifically validated. The method of sperm sexing that effectively sorting X-Y chromosome bearing sperm is flow cytometric that based on the difference in DNA content of X and Y-chromosome bearing sperm. This method is expensive, raised time taking and doubts of potential harmful effects on sperm. Therefore, it is necessary to develop a method of sexing which

is cheaper and easier, especially to application in Indonesia which are limited in terms of flow cytometric ownership. The easier and cheaper sexing method is albumin gradient, one of which is by using bovine serum albumin (BSA).

Bovine serum albumin has been used for sperm sexing media especially for bull semen by making many different levels of BSA in the tube Bovine Serum. Due to a weight basis, albumin is more effective than other commercially macromolecular substances tested, particularly at maintaining levels of stimulated motility. The action of BSA appeared independent of ionic

strength and of common constituents of media. Serum Albumin stimulate sperm motility, and have the ability to prevent the cells sticking to the container surface. Ericsson et al. (1973) was firstly reported separation using an albumin gradient for separation of X and Y-bearing spermatozoa (Sharma and Sharma, 2016). The albumin gradient method was effective to increase proportion of the sperm and resulted good motility and elimination the abnormal sperm (Maxwell et al., 1984)

Differing mass and motility of X and Y chromosome bearing sperm is the principles use of BSA for sperm sexing. It was reported that the Y-chromosome is smaller than the X-chromosome and therefore the X-chromosome takes up more of the DNA-specific stain. Differences in DNA content of X- and Y-chromosome bearing sperm from some animals were reported and in rams is about 4.2 % (Johnson, 2000). Application of sperm sexing in ram still limited. It was reported that the natural proportion of X-Y chromosome bearing sperm in ram's semen is 50,70% X : 49,30% Y (Solihati et al., 2017). On the other hand, research about ram's semen processing had been reported such as level of cryoprotectant (Solihati et al, 2018a), addition of antioxidant (Solihati et al, 2018b). It was reported that concentration of albumin and the albumin concentration differences were factors involve in the successful of sperm sexing (Meistrich, 1982). Nevertheless, the research about combination of BSA concentration as sexing media still limited, especially for ram semen no data was found. This research aims to evaluate the proportion of X and Y chromosome bearing sperm of local ram after sexing with different combination of BSA concentration and to find out the combination of BSA concentration that resulting higher proportion of X and Y chromosome bearing sperm.

## Materials and Methods

Research used ten ejaculates from two local rams, 3 years old. Rams was fed grass and

concentrate. The ejaculate was collected used artificial vagina and were evaluated macroscopic and microscopic. Design of the research used CRD, with four treatments and ten repetitions. Treatment consists of BSA concentration combination at upper and bottom layer, consist of: T1 (3% & 6%), T2 (4% & 8%), T3 (5% & 10), and T4 (6% & 12%). After sexing process, the layers of upper and bottom are separated and added 2 ml solution of Brackett Oliphant (BO) and then to be centrifuged for 10 minutes at 1800 rpm. After that, the pellets are diluted in 1 ml egg yolk tris extender for the semen evaluation according to the observed parameters. Parameter consists of the proportion of X-Y chromosome bearing sperm.

The procedure of calculation for proportion the X-Y chromosome bearing sperm: prepared 1 drop spermatozoa sample from each semen fraction as a result of the treatment using 2% eosin solution; measurement of the length and widest part of the spermatozoa head using a 10 x 100 microscope using a micrometer lens; sperm counted from each fraction of at least 200 cells using the DP2 BSW program for measuring the length and width of the sperm head ; head size that is larger than the average head of fresh semen is categorized as X sperm; a head size that is smaller than the average head of fresh semen is categorized as Y sperm, the proportions of sperm X and Y are calculated.

Data was analyzed using analysis varians to know the effect of treatment, and further analysis used Duncant tes to know the differences between treatments. The data analysis was done to each BSA layer.

## Results and Discussion

The fresh semen that used in this research was evaluated macroscopic and microscopic. The value was showed at Table 1, it showed that the fresh semen have qualify quality for going to sexing sperm. The volume of fresh semen obtained on the macroscopic evaluation was 1.00 - 1.20 ml.

Table 1. Macroscopic and mikroskopik evaluation of fresh semen

Parameter	Mean Value
Volume (ml)	1.14±0.09
Colour	Creamy
Odor	Rancid
Consistensi	Condensed
pH	6.4±0.07
Mass Movement	+++
Total Sperm Concentration (juta sel/ml)	5,852±1,056.3
Motility (%)	85.23±1.93

This is due to report that local ram generally ejaculated semen with a volume of 0.8 - 1.2 ml (Solihati et al, 2018b), and about 0.73-0,74 ml at 25-48 month of age (Solihati et al., 2018c). The colour of semen obtained is normal. Normal semen has a creamy-white colour and a thick consistency (Solihati et al., 2018c). The normal pH of ram semen is 6.0 – 7.5 (Arifiantini, 2012). Solihati et al. (2018a) reported that pH of local ram is 7,22. pH will affect the vitality of sperm (Dethan et al., 2010). The pH obtained has an normal average. The odor of the semen that was caught smelled fishy. Normal ram semen has a distinctive fishy smell and is accompanied by the smell of the animal itself (Arifiantini, 2012; Nahriyanti et al., 2019)). Mass movement, total sperm concentration and motility were evaluated microscopically. The results of the observations on the mass movement of ram semen showed the formation of black, dark, thick and fast-moving cloud waves. The mean total sperm concentration of ram at fresh semen is normal.

This is due to the report that the normal total sperm concentration of ram is 3,500 – 6,000 million cells/ml (Susilawati, 2011). The mean motility of fresh semen was also in normal range. These results are consistent with the research before which stated that the motility of fresh semen from local ram at 25-48 month of age ranges from 81.74 – 87.21% (Solihati et al., 2018c). The sperm motility of Garut ram is 75% (Herdis et al., 2015), and 72.92% (Nalley and Arifiantini, 2013). The proportions of X

chromosomes bearing sperm (X sperm) and Y chromosomes bearing sperm (Y sperm) as a result of the study with several combinations of BSA concentrations (Albumin Column method) showed in Table 2. In local ram, the proportion of X sperm in the upper layer showed differences depending on the BSA concentration or albumin column used. The proportion of X sperm in the upper fraction at 5% BSA produced the highest proportion compared to BSA 3%, 4% and 6%. When compared with other livestock species, the proportion of X sperm in local ram is almost comparable to the proportion of X sperm in the Etawah Cross-breed goat that has been reported by other authors (75.40%) (Solihati et al., 2017b) and the proportion of X-chromosome bearing sperm in dairy cows (71.22%) using the same BSA concentration is 5% (Kaiin et al., 2003). This is due to differences in sperm motility X and Y, so that spermatozoa that have high motility (Y sperm) will be able to penetrate the fraction concentration with a more concentrated BSA concentration, while sperm X will remain in the fraction with a low BSA concentration.

As the BSA concentration increases, it makes more difficult for sperm to penetrate the higher level of BSA concentration. The decrease in proportion of X-sperm that occurs in higher level BSA is due to high concentration of BSA in the bottom layer (12%), which causes the X sperm to become stuck in the upper layer. Differences of motility occurs because the size and weight of the X and Y sperm heads are different. Many researchers before has been quantified the

difference in DNA content between X- and Y-sperm of many animals. The difference in weight and size of the X- and Y-sperm heads is caused by DNA content in sperm head that X-sperm greater than Y-sperm which is 3.8% at bull (Garner, 2006), 3.6% at boar (Lu et al., 2013), 4.2% at ram (Johnson, 2000), 4.4% at buck (Parrilla et al., 2004), and 3.7% at stallion (Johnson, 2000). This difference causes the Y sperm contain fewer genetic material, as a result Y sperm are lighter and have a faster movement than X sperm, making it easier for Y sperm to penetrate the concentrated albumen layer.

The proportion of Y sperm in local ram also showed a difference depending on the BSA concentration or albumin column used. The Y sperm proportion at bottom layer in BSA 10% and BSA 12% resulted in the same proportion, higher than BSA 6% and 8%. In other livestock species such as PO cattle, the proportion of Y sperm using BSA 10% is 76.65% (Kaiin et al., 2003). The combination of BSA concentrations of 5% and 10% produced the highest mean proportion of sperm compared to other combinations of BSA concentrations in both the upper and bottom layers in this research.

In research before, Solihati et al. (2021) found that combination of BSA concentration 5% and 10% evidently showed the almost same of pH (7.43 for 5% BSA and 7.40 10% BSA), this will have a good impact on sperm whereas pH value can give the sperm a comfort condition through treatment of sexing with albumin columns, it because the sperm were not pass the internal pH changes. Contri et al (2013) was reported by that medium pH significantly affected the movement of sperm, and internal pH would be modified by the up and down external pH, whereas the internal pH regulate sperm motility that linked with activity of mitochondria. Sperm motility was clearly affected by the structure and function of mitochondria. Also, at neutral pH the

enzyme at mitochondria active, so pH of sperm decrease will be decreasing motility of sperm.

Chen et al. (2014) reported that hydrogen-ion concentration outside spermatozoa could affect sperm motility and capacitation by influencing sperm Na<sup>+</sup>/K<sup>+</sup>-ATPase activity and spermoplasm Ca concentration. Furthermore, human sperm motility and capacitation was reported by Zhou et al. (2015) were stimulated by alkaline conditions. Acidic environment caused the dramatically decreased Na<sup>+</sup>/K<sup>+</sup>-ATPase activity, that could be the reason of lower sperm motility and Ca<sup>2+</sup> level, which ultimately affected pregnancy. Also, acidic environment may be directly able to damage the sperm cell membrane, or to increase the active oxygen content, thus affecting sperm motility and capacitation, which warrant further explorations. Also, it was reported that BSA concentration of 5% at upper and 10% at bottom layer resulting longest longevity of sperm in post thawed and chilled sexed sperm (Solihati et al., 2022). Base on this report, It could be said that at combination BSA concentration of 5% and 10%, may be the best combination for sperm to move optimally.

According to Majorek et al. (2012), serum albumin most abundant in mammals, is the plasma protein and a multifunctional protein with extraordinary ligand binding capacity, so making it a transporter molecule for a diverse range of metabolites, drugs, nutrients, metals and other molecules. Albumins also have wide applications on clinical, pharmaceutical, and biochemical. Zhang et al. (2015) reported that BSA is a cow serum albumin protein, it efficiently can protect sperm and act as an antioxidant that maintain the sperm quality by protecting sperm plasma membrane from damage. Nevertheless, the use of the albumin column method with a concentration of 12% more is not effective for X and Y sperm separation (Somarny et al., 2011).

Table 2. Proportion of X-Y Chromosom Bearing Sperm at Various Combination of BSA

Concentration		BSA Concentrations			
Proportion	3%	4%	5%	6%	
X-Sperm (%)	54,90±1,63 <sup>c</sup>	60,55±1,59 <sup>b</sup>	75,55±1,09 <sup>a</sup>	61,80±1,97 <sup>b</sup>	
Y-Sperm (%)	54,95±1,50 <sup>c</sup>	60,50±1,97 <sup>b</sup>	76,45±1,12 <sup>a</sup>	79,00±1,15 <sup>a</sup>	

Significantly ( $p < 0.05$ ) different was showed by different superscrip at same row

The proportion of Y sperm tends to increase as the BSA concentration increases. This occurs because the increased BSA concentration causes the difficulty level of X sperm to penetrate the lower fraction of the BSA layer, so that Y sperm will be more in bottom layer because of its higher motility compared to X sperm. The difference in the average proportion of X-Y chromosome bearing sperm caused by the higher concentration of albumin in the buttom layer, the more difficult it is for sperm to penetrate the albumin layer. The higher albumin concentration in the buttom layer, resulting the less sperm in that layer, which caused the sperm was concentrated in the upper layer.

The X- and Y-sperm proportion to be produced from this study still did not meet the expectations that is reach high proportion in both bottom and upper fraction. This possibility is one of the weaknesses of the sexing method with the albumin column method. However, this proportion can increase the chances of obtaining the expected sex compared to the natural proportion, so that under the limited flow cytometry, the albumin column method can be used as an alternative to be sexing method. The addition of certain ingredients in future studies is expected to increase the proportion of X- or Y-chromosome bearing sperm, may be such an antioxidant.

## Conclusions

This is concluded that combination of BSA concentration affect the X-Y chromosome bearing sperm proportion and the combination concentration of 5% and 10% BSA at upper and

bottom layer resulting higher proportion of X and Y chromosome bearing sperm.

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