Viability of ram’s X-Y sperm after sexing with bovine serume albumin at different incubation time

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Abstract. Sperm sexing technology was done to increase reproductive efficiency due to artificial insemination (AI) programme. It can improve the proportion of certain chromosome bearing sperm from its natural proportion, but there is not information about the viability of sexed sperm with Bovine Serum Albumin (BSA) in Local Ram. The aims of the research were to find out the viability of X-Y sperm after sexing with BSA at different incubation time, which one of the incubation time that maintain the longest viability and to find out which kind of sperm that have higher viability. This research was done with completely randomized design with three treatments of incubation time (45, 60, and 75 minute) and eight replications. The sexing method was used is columns of BSA. Parameters that were evaluated were viability of X-sperm and Y-sperm at 0% motility.

Result showed that incubation time significantly effect on X and Y-sperm at 0% motility. X-sperm viability at incubation time 45, 60 and 75 minute were 5.5, 5.0 and 4.33 days respectively at 40% motility, and about 12.38, 11.88 and 11.50 days at 0% motility. Viability of Y-sperm at incubation time for 45, 60 and 75 minute were 4.17, 4.0 and 3.5 days up to 40% motility and 10.75, 10.13 and 9.5 days up to 0% motility. It is concluded that the viability of X and Y-sperm were resulted from 45 minute of incubation time longer than 60 and 75 minute incubation time both of up to 40% and 0% motility, and the X-sperm have highest viability than Y sperm.

1. Introduction
Sperm Sexing technology in livestock is an effort to increase the chances of certain sexes birth in accordance with the purpose of livestock raising. The application of sperm sexing technology can improve the efficiency of Artificial Insemination (AI) programme.

One of the sperm sexing technique is the column albumin technique, by making several layers or different fractions of albumin solution. This technique is based on the discovery that there are differences between X-chromosome bearing sperm (X-sperm) and Y-chromosome bearing sperm (Y-sperm). It is known that X-sperm has a larger head size but has a slower movement than Y-sperm. On the basis of these findings, an albumin column is made in a tube with a higher concentration placed at the bottom of the tube and a lower concentration at the top of the tube. Y-Sperm has a faster movement so it will be able to penetrate a thicker layer, so the majority of Y-sperm will be obtained in the lower layers/fractions wherein X-sperm will be obtained in upper layer/fraction
Regarding the reproductive performance of Local Sheep has been previously reported among others relating to the quality of semen of Local Sheep at puberty age [1], age of Local Sheep which produces the best quality semen [2], use of cryoprotectants for frozen semen Local Sheep [3] [4]. The research about sperm sexing on Local Ram semen have done before, whereas the natural proportion of X-Y sperm was 50.70% : 49.30% [5], but the comparison of X and Y sperm accordance with these viability has not been reported before. The aim of the research was to find out the viability of X-Y sperm after sexing with Bovine Serum Albumin (BSA) at different incubation time, to find out the incubation time that maintain the longest viability and which kind of sperm that have the longer viability.

2. Material and method

The research used Local Ram, 3 years of age, was fed by forage and concentrate, and caged individually. Semen was collected by artificial vagina and evaluated macroskopic and microskopic.

2.1. Materials

The main materials was Bovine Serum Albumin (BSA), egg yolk tris extender, and BO (Bracket-Oliphant) medium, antibiotic, eosin. The equipment to be used were tube, rack, micropipette, waterbath, sentrifuge, glassware, microscop, counter.

2.2. Sexing method

Sexing method used bovine serum albumin (BSA) columns. BSA was diluted with BO medium and was made for two different layer (10% for bottom layer and 5% for upper layer). The bottom and upper layer BSA entered the tube for 2 ml. Fresh semen was diluted by BO medium with comparison 1 semen : 4 BO, and then 1 ml of diluted semen was entered to the tube and then going on incubation time at waterbath with 37°C in temperature. After that the upper and bottom layer was separated and placed into others tube, and the going on centrifuge at 1800 rpm for 10 minute. After that, pellet was diluted by egg yolk tris extender and then going on equilibration time for three hours. The evaluation of viability was started after equilibration time and repeated every 24 hours up to 40% and 0% motility.

2.3. Research design and data analysis

This research was design by completely randomized design with three treatments and eight replication. The datas were analysed using analysis of varians (ANOVA) and Duncan test. The treatments consist of three incubation time (45, 60, and 75 minute). Incubation time is time for sexing itself. The parameters consist of the viability of sexed semen from bottom and upper layer up to 40% and 0% motility.

3. Result and discussion

The viability of Local ram sexed sperm was evaluated at 40% and 0% motility. Result of ANOVA showed that incubation time significantly effect on viability of X and Y-sperm only at 0% motility, not for 40% motility. This result showed that Y-sperm at bottom layer had shorter viability than X-sperm at upper layer for all treatment of incubation time both of at 40% and 0% motility.

Viability up to 40% motility showed at figure 1. Sexed sperm at upper layer had viability longer than bottom layer. Sexed sperm at upper layer was dominated by X-sperm and sexed sperm at bottom layer was dominated by Y-sperm. Result showed that viability up to 40% motility for X-sperm at incubation time 45, 60 and 75 minute were 5.5, 5.0 and 4.33 days resfectively, Y-sperm viability were 4.17, 4.0 and 3.5 days. Result of ANOVA showed that incubation time not significantly effect on viability of X and Y-sperm at 40% motility, although the shorter incubation time seem resulting longer viability.

The research also showed that up to 40% motility the X-sperm resulting longer viability than Y-sperm in all incubation time. It can be explained that X-sperm have slowly movement because of its
head width, it would not pass thicker layer and so it can save more energy and keep the membrane intact than Y-sperm.

![Figure 1](image)

**Figure 1.** Viability of sexed sperm up to 40% motility at upper and bottom layer

In chilled semen, storage conditions over a long period of time cause a decrease in the quality of spermatozoa due to lactic acid resulting from cell metabolic processes which cause the condition of the medium to become more acidic. Decrease in motility caused by incubation time and centrifugation process increasing the production of Reactive Oxygen Species (ROS) and then ROS will react with unsaturated fatty acids found in the cell membrane through oxidation reactions namely lipid peroxidation. Oxidative stress and stress on ROS can result in damage to cell membrane integrity and decreased sperm motility [6]. This condition can be toxic to spermatozoa which eventually causes sperm death [7]. Lactic acid levels can shorten the survival of spermatozoa [8].

The sperm viability on this research can predict how long the sperm life time in chilled semen after sexing. It usefull for the inseminators in order to AI time for Local Ram. The motility was recommended for AI is not less than 40%.

Viability up to 0% motility showed at figure 2. Sexed sperm at upper layer was dominated by X-sperm and sexed sperm at bottom layer was dominated by Y-sperm. Viability up to 0% motility for X-sperm at incubation time 45, 60 and 75 minute were about 12.38, 11.88 and 11.50 days, and for Y-sperm were 10.75, 10.13 and 9.5 day. In upper layer, it showed that X-sperm viability decreased in line with increasing incubation time. ANOVA test at 0% motility showed that incubation time significantly effect on viability, but Duncan test showed that 45 minute incubation time was not significantly different with 60 minute and 60 minute was also not significantly different with 75 minute, viability of 45 minute significantly longer than 75 minute.

Viability is closely related to the sperm motility because the sperm viability is known by measuring the duration of decreasing sperm motility which moves progressively until there is no more movement. The X sperm viability from 45 minute to 75 minute has decreased. The motility after 30 minutes separation (76.4%) was lower than 20 minutes (77.5%) and 10 minutes (79.4%) [9], this research showed that the metabolism increase in line with incubation time so that it will decrease sperm motility. Research on sperm of Bali cattle with treatment of sexing time differences of 20, 35, and 50 minutes. In the upper layer, treatment time of 20 minutes has the higher percentage value (68.50 ± 24.72%) compared to 35 minutes (66.25 ± 23.57%) and 50 minutes (65.00 ± 23.19% ). In general, it can be said that the longer the sexing time, the lower the percentage of sperm motility [10].
Y-sperm in bottom layer have smaller, lighter and shorter heads compared to X-sperm, so Y-sperm move faster, and then will be followed by faster death than X-sperm, and X-sperm are more viable because its energy was efficient [9].

The average sperm motility of the bottom layer is lower than the upper layer. This is because of the sperm at bottom layer has passed through two layers, so that the energy was used more consequently and then will reduce motility or even not move at all. Sperm storage for a long time will cause a decrease the sperm quality due to the presence of lactic acid resulting from cell metabolic processes which results in an increasingly acidic medium condition [11].

The research also showed that up to 0% motility the X-sperm resulting longer viability than Y-sperm in all incubation time.

Figure 2. Viability of Sexed Sperm up to 0% Motility at Upper and Bottom layer.

The results of this study prove that sexing sperm using BSA columns with different concentrations makes the sperm with higher speeds penetrate the more concentrated BSA media (bottom layer). Sperm in the bottom layer have a higher motility compared to sperm in the upper fraction. This causes the sperm in the lower fraction to spend more energy on their movements. The more movement in the sperm, the more energy was released by the sperm so that the sperm will run out of energy and the viability decreases during storage. This happens due to decreased nutrien substances for sperma and an increase in sperm dead. Dead sperm can be toxic to living sperm so that their quality generally decreases. Another factor that causes a decrease in sperma viability is storage temperature. Sperm stored at 5°C will experience a decrease in motility and viability [12].

Sperm sexing process can triggered plasma membrane damage as result from lipid peroxidation. This process haven because of increasing the ROS production that imbalances with antioxidant capacity in sperm [13]. ROS molecule of sperm fisiologically involved in fertilization process, but at overload level was dangered for sperm. Sustainable of peroxidation process resulting high damage of sperm, decrease the fluidity and increasing permeability on certain ions [14].

Membrane damage occure during sperm sexing process, which sperm at bottom layer (Y-sperm) have faster movement capability than sperm at upper layer (X-sperm), so that sperm at bottom layer could passed two layers of BSA until higher BSA concentration [15]. According to that statement, it can be said that the higher concentration of BSA, of course have higher viscosity and density, so that the friction experienced by sperm in the lower layer increased. This friction force causes high damage to the membrane to the lower layer sperm. In addition, during the cryopreservation process occurs the formation of ice crystals (cold shock) which can cause damage to the sperm cell membrane.
Temperature changes during the cryopreservation process and thawing of sperm cells triggers changes in permeability and functional membranes that cause various sperm damage [16, 17]. Another factor that causes greater membrane damage is the faster movement of sperm in the lower fraction, so that the level of metabolism is higher, and the ROS produced is greater than the sperm in the upper fraction [18]. Hydroxyl radicals (-OH) are one of the metabolic ROS molecules with very high reactivity and can initiate lipid peroxidation. The lipid peroxidation process occurs through two mechanisms, which are influenced and without influenced by hydroxyl radicals [19]. The sperm cell membrane contains PUFA which is susceptible to ROS, during the lipid peroxidation process almost 60% of the fatty acid content is lost from membrane phospholipids. This certainly causes changes in the integrity of the sperm cell membrane and increases the permeability of the membrane, so that certain substrates or ions easily enter into sperm cells [14].

4. Conclusion
The viability of X and Y-sperm were resulted from 45 minute of incubation time longer than 60 and 75 minute incubation time both of up to 40% and 0% motility, and the X-sperm have highest viability than Y sperm.

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