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Quality and Longevity of Local Ram's Sexed Sperm with Albumin Column

Nurcholidah Solihati[1,a], Siti Darodjah Rasad[1], Annisaa Yusrina[1], Kikin Winangun[1], Toha[1]
[1]Faculty of Animal Husbandry Universitas Padjadjaran; Jl Raya Bandung Sumedang Km 21 Jatinangor Sumedang
[a]e-mail: nurcholidah@unpad.ac.id

Abstract

The aims of the research were to determined the sperm quality and longevity after sexing with albumin column. This study used 10 ejaculate semen from two local sheep around 2-3 years old, and used 5% and 10% of bovine serume albumin. The treatment were incubation time (45, 60 and 75 minutes) with 10 replication. Parameters consist of sperm motility, abnormality, intact plasma membrane (IPM), intact acrosome cup (IAC) and longevity. Data were analyzed by analysis of variance and Duncan's test. The results showed that both at upper and bottom fraction, incubation time significantly ($p<0.05$) effect on motility, IPM and IAC. At upper fraction incubation time for 75 minute obtained the highest motility (71.24%), IPM (67.70%) and IAC (68.85%), nevertheless at bottom fraction the highest motility (73.72%), IPM (72.95%) and IAC (72.05%) were obtained at 45 minute incubation time. The result also showed that incubation time for 45 minute obtained the longest longevity both at upper (7.5 days) and bottom (7.0 days) fraction. It is concluded that the quality of sexed sperm of local ram fulfilled the quality for artificial insemination and the incubation time for 45-75 minutes can be used to obtain the qualify sexed sperm for artificial insemination with chilled semen.

Keywords: sperm sexing, albumin colums, incubation time

Kualitas dan Daya Tahan Hidup Sperma Domba Lokal Hasil Sexing dengan Kolom Albumin

Abstrack

Tujuan penelitian ini untuk mengetahui kualitas dan daya tahan hidup sperma setelah sexing dengan kolom albumin. Penelitian ini menggunakan 10 ejakulat semen dari dua domba lokal berumur sekitar 2-3 tahun, dan menggunakan bovine serume albumin 5% dan 10%. Perlakuan adalah lama inkubasi (45, 60 dan 75 menit) dengan 10 ulangan. Parameter terdiri dari motilitas, abnormalitas, membran plasma utuh/MPU, dan tudung akrosom utuh/TAU dan daya tahan hidup sperma. Data dianalisis dengan analisis varian dan uji Duncan. Hasil menunjukkan bahwa baik pada fraksi atas maupun fraksi bawah lama sexing berpengaruh nyata ($p<0.05$) terhadap motilitas, MPU dan TAU. Pada fraksi atas lama inkubasi selama 75 menit menghasilkan motilitas tertinggi (71.24%), MPU (67,7%) dan TAU (68,85%). Namun pada fraksi bawah motilitas tertinggi (73,72%), MPU (72,95%), dan TAU (72,05%) diperoleh dari lama inkubasi selama 45 menit. Hasil juga menunjukkan bahwa lama inkubasi selama 45 menit mencapai daya tahan hidup terlama baik pada fraksi atas (7,5 hari) maupun fraksi bawah (7,0 hari). Disimpulkan bahwa kualitas sperma domba lokal hasil sexing dengan kolom albumin memenuhi kualitas untuk inseminasi buatan dan lama inkubasi selama 45-75 menit dapat digunakan untuk memperoleh sperma hasil sexing yang memenuhi syarat kualitas inseminasi buatan dengan chilled semen.

Kata kunci: sexing sperma, kolom albumin, lama inkubasi

Introduction

Sperm sexing is a method of separation between X chromosome bearing sperm (X-sperm) and Y chromosome bearing sperm (Y sperm) to obtain a higher proportion of certain sperm, the hope of getting a higher probability of birth. One of the easy ways to use sexing methods is the albumin column

method using bovine serum albumin (BSA). The method is based on the discovery of sperm head area, head length and DNA content and sperm movement between X-sperm and Y-sperm, in which Y-sperm has a smaller and lighter head area than X-sperm, nevertheless Y-sperm have shorter head length with fewer DNA and faster movements than X-sperm. The success of sexing method with albumin column can be influenced by several factors, one of which is the length of incubation time, it mean the length of the sexing process itself. Different incubation times are thought affect to quality and longevity between X sperm and Y sperm, but information about this is still limited. Some researchers have performed sperm sexing with a 60-minute incubation period (Kain et al., 2015, Hadi and AL-Tamimi, 2017), and faster incubation times for 20, 35 and 50 minutes (Sunarti et al, 2016), and 10 , 20, 30 minutes (Situmorang et al, 2013), and 30, 60, 90, 120 minutes (Afriani et al, 2011). Research on local ram's semen is eligible for processing into chilled semen (Solihati et al., 2016) and frozen semen (Solihati et al., 2018) for use in artificial insemination (AI) programs. The local ram has sperm head areas ranging from 22.97 - 59.30 μm^2 with a natural proportion of sperm of 50.70% (X) and 49.30% (Y) (Solihati et al, 2017). Information about the quality and sperm longevity which is resulted from sperm sexing will be useful for the AI program especially in ram that is about sexing time that reach the best semen quality and longevity. The aim of the research was to knew the quality and longevity of local ram's sexed semen with albumin column.

Materials and Methods

This study used 10 ejaculate semen from two local ram aged 2-3 years. Semen was collected by the artificial vaginal method. The study was conducted by laboratory experiment using a completely randomized design with three treatments incubation times (45, 60 and 75 minutes) and repeated 10 times.

The sexing method used was a two layers bovine serum albumin (BSA) column, with a 5% BSA concentration at the upper layer and 10% at the bottom layer. Bovine serum albumin is composed of a single polypeptide chain which includes 583 amino acids residues (Roufegarinejad et al, 2018). The incubation time is according to the treatment given, as the same as sexing time. After sexing process, the upper and bottom layers are separated and added Brackett Oliphant (BO) solution to be centrifuged for 10 minutes at a speed of 1800 rpm. The pellets are diluted with egg yolk tris extender for the semen to be evaluated by making preparations according to the observed parameters. The parameters consisted of semen quality (motility, abnormalities, intact plasma membrane, intact acrosome cup) and sperm longevity after sexing. The sperm abnormality were evaluated by differential preparat using eosin-nigrosin staining. The intact plasma membrane (IPM) was evaluated using hypoosmotic swelling (HOS) test. The HOS solution was made by 0,179 gr NaCl with 100 ml distilled water. The HOS test was performed by incubating semen and HOS solution at 37 °C for 30 minutes. The swelled and coiled sperm was identified as an intact sperm, while the straight sperm was identified as a non-intact sperm. Total cell number was performed on 200 cells. The Intact acrosome was observed by observing the condition of the sperm head perfection using a microscope at 10x40 magnification. 100 ml of physiological NaCl solution and 1 mL of formalin are mixed until homogeneous. Then 0.1 ml semen was added 0.2 ml of a mixture of formaldehyde and physiological NaCl to be prepared as a review preparation. The sperm longevity was evaluated by sperm motility until 40%.

The data obtained was processed by analysis of variance and post hoc test using Duncan's

advanced test. Data processing used SPSS version 19.

Results and Discussion

Quality of Sexed Sperm with Albumin Column in Several Incubation Times

The quality of sexed sperm observed in this study included motility, abnormalities, intact plasma membrane (IPM), and intact acrosome cup (IAC). The results showed that in the upper layer (Table 1) the treatment of incubation time had a significant effect on motility, IPM, IAC, but did not significantly affect abnormalities. Duncan's test showed that in the upper layer, incubation time of 45 minutes resulted in motility (75.16%) equal to the incubation time of 60 minutes (73.18%), but the incubation time of 45 minutes resulted in the highest MPU, TAU values. Based on the results of this study it can be said that the incubation period for 45 minutes produced the best sperm quality, however, the semen quality both of them still met the eligibility requirements for use in artificial insemination (AI) programs using chilled semen. The results showed that in the bottom layer (Table 2) the treatment of incubation time had a significant effect on motility, IPM, IAC, but did not significantly affect on abnormalities. The result showed that the incubation time of 45 minutes resulted in the same motility with incubation time of 60 minutes, but 45 minutes produced the highest IPM, IAC.

The sexed sperm motility of this study experienced a slight decrease This is due to the sexed semen having undergone an incubation process, and washing the semen by centrifugation which results in damage of sperm tail, so that its movement will decrease. The detrimental effect of separating plasma semen by centrifugation is the increase in the formation of reactive oxygen species (ROS) by spermatozoa. According to Agarwal et al. (2003), increased production of ROS by sperm after centrifugation is thought to be a complex process and can originate from various chemical processes, which occur in organelles and cells or originating from outside the cell. Various biological processes that can be modulators of ROS formation by spermatozoa include modulator of mechanical damage to the sperm membrane and the separation of seminal plasma from spermatozoa (Iwazaki and Gagnon, 1992). Seminal plasma is a medium for spermatozoa consisting of secretions from the testes, epididymis, and accessory glands (Manjunant et al, 1997). The mixture consists of several factors such as organic and inorganic materials which are important in the mechanism of maturation of spermatozoa under the control of hormones and enzymes (Dogan et al, 2009).

The decrease in motility after the sexing process is due to sperm exposure and must penetrate the sexing media, namely the albumin layer which depletes sperm energy. In addition, the washing process results in a reduction in the plasma semen concentration and replacement of the solution with Brackett Oliphant (BO) medium is possible as one of the factors that causes a decrease in the value of sperm motility.

The decrease in motility caused by the incubation period and the centrifugation process that might affect the level of ROS production. Reactive Oxygen Species will react with unsaturated fatty acids contained in cell membranes through oxidation reactions, namely lipid peroxidation. Oxidative stress and stress on ROS can cause damage to cell membrane integrity and decrease in sperm motility (Henkel, 2004).

The value of intact plasma membrane in this study decreased in order with increasing incubation time. This is because of increasing in sperm movement and metabolism as the addition of incubation time. The results of this study also showed that there was a decrease in the intact plasma membrane value of sperm resulting from sexing

compared to fresh sperm. This is consistent with the report of Gardner (2006) that the sexing process has an effect on the integrity of the membrane where the changes are related to mechanical stress.

Sperm with an intact membrane has a positive influence on motility and viability. This is because the metabolic process runs well so that the spermatozoa receive an energy supply in the form of ATP which is sufficient for its movement. Sperm with intact membranes are able to regulate the entry and exit of nutrients (substrate and electrolyte) which are needed in the metabolic process to and from the cell. It could be said that plasma membrane integrity is a condition that shows the physiological function of the membrane which is maintained against the transport of water so that the transport process runs stably.

If the plasma membrane damaged, the sperm metabolism will be disrupted. Synthesis of ATP will run abnormally and decrease the sperm motility and longevity. The membrane integrity has important functions in metabolic processes, capacitation, acrosome reactions, and binding of sperm to the surface of the oocyte (Baqir et al, 2009). The sperm plasma membrane integrity can be damaged if the presence of toxic substances both of dead sperm and substances contained in diluents which have undergone oxidation, because the storage can cause high levels of free radicals. Plasma membrane integrity is a prerequisite for the survival of sperm (Sharma et al, 2011). If the plasma membrane has been disrupted or damaged, it will result in aniosmotic conditions that cause intracellular leakage, which will affect ATP reshuffle and thus affect sperm motility (Bohlooli et al, 2012).

The good plasma membrane integrity of sperm shows that phospholipids can survive and maintain well against any crash between the tube and medium when sexing. Phospholipids function to maintain membrane integrity and form a dynamic surface between cells as a protection against environmental conditions. The process of sexing by centrifugation can cause partial release of sperm membrane phospholipids due to mechanical effects, namely the presence of centrifugal force. The release of a portion of membrane phospholipids can cause membrane integrity to be disrupted and thus affect the sperm viability. The sperm viability can affect the motility and sperm membrane integrity so that motile sperm do not have good membrane integrity (Diliyana, 2014).

The incubation time did not significantly affect abnormalities in the upper fraction. This is because when the incubation process, the upper fraction sperm do not experience many crash or friction between other sperm, because this incubation principle is to allow the sperm to penetrate the fraction of different concentrations, with its own motile power, so the risk of damage to the sperm head was not high. Moreover, the incubation time have no significant effect on the sperm abnormalities. This result corresponding with Kaiin et al. (2013) that reported the sperm sexing with BSA columns tend to decrease sperm abnormality because of sperm selection, only the motile sperm could penetrate the BSA columns, while abnormal sperm stay on the surface of BSA columns. Sperm abnormalities resulting from sexing in this study ranged from 3.05% - 3.65%. This values still in the normal range because it does not exceed 20%. This is in accordance with Ihsan (2009) which explains that semen which can be used by AI has sperm abnormalities should not exceed 20% and if sperm abnormalities of more than 20% will reduce fertility. Also, Ariantie et al. (2013) explained that semen with a high percentage of abnormalities tended to have low fertility because it was related to the ability to initiate fertilization or maintain embryonic development.

The results of sperm acrosome integrity in the upper fraction decreased with increasing incubation time. This happens because sperm have experienced physical and chemical disorders during the incubation process. Damage to the acrosome cup is also caused by the centrifugation process during the sexing process. This is in accordance with Susilowati (2010) that there was a decrease in the quality of spermatozoa after centrifugation at a speed of 1800 rpm for 10 minutes. The sperm intact acrosome cup of fresh semen in goats as much as 84.00 ± 4.4% but after centrifugation at a speed of 1800 rpm for 10 minutes decreased the intact acrosome cup to 59.00 ± 4.25. The results of this study are in accordance with Sianturi et al., (2007) that the percentage of sperm with intact acrosome cup decreased with increasing incubation time. Burroughs (2011) reported that BSA is able to bind sperm plasma membranes and adsorb cholesterol by BSA molecules. Damage to the plasma membrane will cause loss of motility and fertilization of sperm, due to the release of cellular components and inactivation of important enzyme proteins in the acrosome. Acrosome cup play an important role in the fertilization process because their enzymes are needed to penetrate the cumulus oophorus and pellucida zone.

Carvalho et al (2010) reported the results of in vitro studies that changes in sperm quality did not affect the power of fertilization and blastocyst formation from sexing sperm. The use of sexing sperm did not reduce the rate of embryo development compared to the use of non-sexing sperm. The same was reported by Underwood et al (2010) and Peippo et al (2010) that there were no differences in the value of blastocyst formation between sperm sexing and non-sexing. Next Carvalho et al (2010) detected clearly the effect of sperm sexing on sperm quality but not on embryo development. This is probably due to decreased motility, membrane integrity and acrosome integrity caused by the sexing procedure is not critical for in vitro conditions compared with in vivo.

Table 1. Sexed Sperm Quality with Albumin Column at Different Incubation Time (Upper Fraction)

Parameters	45 Minute	60 Minute	75 Minute
Motility (%)	75.16±2.03 (a)	73.18±1.25 (a)	71.24±2.90 (b)
Abnormality (%)	3.05±0.86 (a)	3.20±1.23 (a)	3.65±0.53 (a)
Intact Plasma Membrane (%)	74.55±2.01 (a)	70,80±3.90 (b)	67.70±4.87 (c)
Intact Acrosome Cup (%)	74.00±2.46 (a)	71,90±3,48 (b)	68.85±2.91 (c)

Different superscrif at the same row showed significant at P<0.05

Table 2. Sexed Sperm Quality with Albumin Colums at Different Incubation Time (Bottom Fraction)

Parameters	45 Minute	60 Minute	75 Minute
Motility (%)	73.72±2.12 (a)	71.13±2.52 (a)	68.40±3.93 (b)
Abnormality (%)	3.40±1.13 (a)	3.40±1.23 (a)	3.20±0.75 (a)
Intact Plasma Membrane (%)	72.95±2.75 (a)	68.90±4.65 (b)	65.75±5.11 (c)
Intact Acrosome Cup (%)	72.05±4.57 (a)	69.55±3.59 (b)	66.90±3.74 (c)

Different superscrif at the same row showed significant at P<0.05

Aini et al (2016) reported that the sexing spermatozoa with BSA gradient method had the ability to fertilize and support the same embryo development with non-sexing spermatozoa. Kaiin et al (2017) reported the results of his research that the use of BSA during the sexing process did not affect the capacitation status of post-thawed frozen sexing results before being capacitated. The sexing process of sperm using a multi-level BSA column did not cause an early acrosome reaction.

According to this study we reported that sperm quality after sexing with albumin column until 75 minute of incubation time still resulting good quality to fulfillment the minimum quality for AI application with chilled semen. We could found these result both of in upper layer and bottom layer.

Longevity of Sexed Sperm with Albumin Column in Several Incubation Times

The results of the study on sperm longevity are shown in Table 3. Based on the results of the study, it was shown that both the upper and bottom layers, the incubation duration had a significant effect on sperm longevity. The Duncan's test showed that the incubation time for 45 minutes markedly produced the longest sperm longevity compared to the incubation period of 60 minutes and 75 minutes, however, all of them still met the eligibility requirements for use in artificial insemination programs with chilled semen. This information can be used as a reference for the implementation of AI program using chilled semen, so that the farmer can determine the timing of the AI using sexed sperm with the qualify quality.

The sperm longevity in the upper and bottom layers of this study has decreased in order with increasing the incubation time, this is in accordance with the study of Situmorang et al. (2013) that the percentage of motility after 30 minutes separation (76.4%) was lower than with 20 minutes separation (77.5%) and 10 minutes (79.4%). This is due to the increase in incubation time will increase the metabolism and then will reduce sperm motility. Sunarti, et al. (2016) reported a study on Bali Cattle sperm with the treatment of incubation time of 20, 35, and 50 minutes, where the treatment of incubation time did not affect the sperm motility in both the upper and bottom layers. In the upper layer, the treatment time for 20, 35 and 50 minutes resulted in motility of 68.50 ± 24.72%; 66.25 ± 23.57% and 65.00 ± 23.19% respectively, thus it can be said that the decreasing of sperm motility correlated with additional of incubation time. The sperm motility depends on mitochondrial function. Adenosine Tri Phosphate (ATP) is produced by oxidative phosphorylation in the mitochondrial membrane and transferred to microtubules for contraction of the fibrils in the principle piece and end piece of the sperm tail for the the sperm movement.

Table 3. Sexed Sperm Longevity with Albumin Column at Different Incubation Time (Upper Fraction)

Group	45 Minute	60 Minute	75 Minute
Sperm Longevity at Upper Fraction (days)	7.500.53 (a)	7.400.70 (b)	6.900.74 (c)
Sperm Longevity at Bottom Layer (days)	7.000.82 (a)	7.000.47 (b)	6.500.71 (c)

Different superscrif at the same row showed significant at P<0.05

The condition of semen storage for a long time causes a decrease in the sperm quality due to the presence of lactic acid as a result of cell metabolism which causes the condition of the medium to become increasingly acidic. According to Bearden and Fuquay (2000), the level of lactic acid is significantly correlated with the sperm motility. This can shorten the sperm longevity. Similarly, very low temperatures resulting the damage of vital substances of sperm so that intracellular enzymes, lipoprotein, ATP, and intracellular potassium are reduced (Sukmawati et al. 2014). Results will decreased the sperm longevity. The loss of sperm longevity is unavoidable because during the processing of fresh semen into frozen semen, sperm experiences extreme changes in environmental conditions.

Conclusion

Based on the results of the research and discussion, it is concluded that the quality and longevity of local ram's sexed sperm with albumin column were qualify and fulfilled the quality for artificial insemination. It is suggested that the length of incubation/sexing time for 45-75 minutes could be used to obtain qualify sexed sperm quality and longevity for artificial insemination with chilled semen.

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