The Longevity of Sexed Sperm after Sexing with Several Combination of Bovine Serum Albumin (BSA) Concentration

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ABSTRACT

The aim of the research was to evaluate the longevity of sexed sperm of local ram after sexing with different combination of Bovine Serum Albumin (BSA) concentration in chilled and post thawed sexed sperm. The object of the research was 10 ejaculated semen from 3 years old local ram. The method was a Completely Randomize Design (CRD) with four treatments combination of BSA concentration on upper layer and bottom layer (T1: 3% and 6%, T2: 4% and 8%, T3: 5% and 10%, dan T4: 6% and 12%), each treatment was repeated 10 times. Result showed that there is significant difference ($P<0.05$) in sperm longevity both in chilled and post thawed sexed sperm. Sperm longevity in chilled sexed sperm on of upper layer in T1, T2, T3 and T4 were 229.60±16.13, 231.90±10.27, 265.40±15.01, and 196.60±19.21 hours, respectively, as well as on bottom layer in T1, T2, T3 and T4 were 207.70±31.18, 207.90±31.47, 239.10±30.82 and 181.60±31.81 hours, respectively. Sperm longevity in post thawed sexed sperm on upper layer in T1, T2, T3 and T4 were 142.80±2.68, 142.20±1.64, 147.00±3.00, and 142.20±1.64 hours, respectively, and then on bottom layer were 121.80±2.68, 120.60±3.29, 125.40±3.29 and 118.80±1.64 hours, respectively. In conclusion, the combination of BSA concentration of 5% and 10% at upper and bottom layer resulting longest sperm longevity both in chilled and post thawed sexed sperm.

Keywords: *Longevity, Sexed sperm, Albumin.*

1. INTRODUCTION

The use of BSA for sperm sexing based on principles of differing mass and motility of X and Y chromosome bearing sperm. The X chromosome is larger than the Y and therefore takes up more of the DNA-specific stain. The successful separation of X and Y-bearing spermatozoa using an albumin gradient was firstly reported Ericsson et al. (1973) [1]. The method was effective in increasing the proportion of spermatozoa with motility and elimination of abnormal forms [2].

Bovine Serum Albumin is a cow serum albumin protein that can protect sperm efficiently and can act as an antioxidant that maintain the quality of sperm by protecting the plasma membrane from damage due to free [3]. Albumin was more effective to maintaining stimulated motility levels than other commercially available macromolecular substances, and its action appeared independent of ionic strength and of common constituents of media. Serum albumin have ability both to stimulate sperm motility and to prevent the cells sticking to the container surface. Sperm sexing methods was carrying a female (X) or male (Y) chromosome with the BSA column, it was reported resulting 74.05% of X-sperm in Etawah crossbreed goat [4].

It was reported that the successful of sperm sexing depend on albumin concentration and differences of albumin concentration [5]. The characteristic of pH, density and viscosity of several concentration of BSA was reported [6]. In this research the use of BSA for sperm sexing media by making two layers with different concentration of BSA as combination in the upper and bottom layer of the tube. The purpose of the research

was to find out the sperm longevity after sexing with several combination of BSA concentration.

2. MATERIALS AND METHODS

The object of this research was ten ejaculates of local ram, was collected used artificial vagina. The research used completely randomized design with four treatments and 10 replications. These ejaculates were evaluated macroscopic and microscopic. The treatment is the combination of BSA concentration, consists of four combinations of BSA combination at upper and bottom layer consist of: T1 (3% BSA at upper layer and 6% BSA at bottom layer), T2 (4% BSA at upper layer and 8% at bottom layer), T3 (5% BSA at upper layer and 10% BSA at bottom layer), and T4 (6% BSA at upper layer and 12% BSA at bottom layer).

The procedure of sperm sexing: BSA solutions with various concentrations were put into test tubes with a ratio of 2:2 ml. As much 1 ml semen sample was put into a test tube which already contained a column of sperm separation medium and allowed to settle. Incubated sperm for 45 minutes at a temperature of 37-38˚C. After completion of incubation, each sperm fraction was taken up as much as 1 ml using a micropipette and discarded. Then the sperm was washed by adding 5 ml of BO solution in each fraction. Sperm centrifugation was carried out at 1800 rpm for 10 minutes to obtain a clean sperm deposit from the separation medium to obtain a pellet. The pellet was then diluted using 1 ml egg yolk tris containing the antibiotic penicillin at a dose of 1000 IU/ml diluent and streptomycin at a dose of 1 mg/ml diluent. Sperm evaluation was carried out after the sperm pellet was diluted.

The parameter was the sperm longevity after sexing in chilled semen and post thawed semen both of in upper and bottom layer. Sperm longevity is sperm survival until sperm death or motility 0%. In this research, the sperm longevity was evaluated by the sperm motility every 12 hours and intensively every hour if the motility has reached under 40% until sperm died. The evaluation of motility used Neubauer chamber. In this research, the sperm longevity is expressed in hours.

3. RESULTS AND DISCUSSION

Result showed that the fresh semen had good quality, with total sperm concentration ranged from 455 – 698 x 10^7 cells/ml (585.20±1.93 x 10^7 cells/ml), the average of motility was 85.23±1.93%, and abnormality was 3.10±0.22%. The average of DNA fragmentation was 1.70±0.45%, intact plasma membrane (IPM) was 87.3 ± 1.78% and the intact acrosome cap (IAC) was 88±2.20%. The ratio of X-Y sperm resulted from this research were about 54.90% until 75.55% for X-sperm at upper fraction and about 54.95% until 79.00% for Y-sperm at bottom fraction. The highest proportion of X-sperm was resulted from 5% BSA (75.55%) at upper fraction, and the highest Y-sperm was resulted from 10% (76.45%) and 12% BSA (79.00%) at bottom fraction. We found no significant different between 10% BSA and 12% BSA in resulting Y-sperm proportion.

Result showed that there is significant difference ($P<0.05$) in sperm longevity both in chilled and post thawed sexed sperm. Sperm longevity in chilled sexed sperm on of upper fraction in T1, T2, T3 and T4 were 229.60±16.13, 231.90±10.27, 265.40±15.01, and 196.60±19.21 hours, respectively, as well as on bottom fraction in T1, T2, T3 and T4 were 207.70±31.18, 207.90±31.47, 239.10±30.82 and 181.60±31.81 hours, respectively (Table 1).

From the Table 1 it showed that BSA concentration significantly ($P<0.05$) effect on sperm longevity both in upper and bottom layer. Also, there are significant different between the treatments, whereas the concentration 5% at upper layer and 10% at bottom layer of BSA produced the longest sperm longevity than others combination of BSA concentration.

Sperm longevity in post thawed sexed sperm on upper fraction in T1, T2, T3 and T4 were 142.80±2.68, 142.20±1.64, 147.00±3.00, and 142.20±1.64 hours, respectively, and then on bottom fraction were

Table 1. Sperm longevity in chilled semen at upper and bottom layer

Sperm Longevity (hours)	BSA Concentrations			
Upper Layer	3%	4%	5%	6%
	229.60±16.13^b	231.90±10.274^b	265.40±15.01^c	196.60±19.21^a
Bottom layer	6%	8%	10%	12%
	207.70±31.18^a	207.90±31.47^a	239.10±30.82^b	181.60±31.81^a

Table 2. Sperm longevity in post thawed semen at upper and bottom layer

Sperm Longevity (hours)	BSA Concentrations			
Upper Layer	3%	4%	5%	6%
	142.80±2.68^a	142.20±1.64^a	147.00±3.00^b	142.20±1.64^a
Bottom layer	6%	8%	10%	12%
	121.80±2.68^b	120.60±3.29^b	125.40±3.29^c	118.80±1.64^a

121.80±2.68, 120.60±3.29, 125.40±3.29 and 118.80±1.64 hours, respectively (Table 2).

From the Table 2, in post thawed sexed semen it showed that BSA concentration also significantly ($P<0.05$) effect on sperm longevity both in upper and bottom layer. There are significant different between the treatments, in which the concentration 5% of BSA at upper layer and 10% at bottom layer produced the longest sperm longevity than others combination of BSA concentration of post thawed sexed semen.

The reason to explained this research result is about the pH of the BSA concentration. It was reported that the combination of BSA 5% and 10% whose the normal pH that suitable for sperm life. [6]. The value of pH from 5% and 10% BSA concentration was surprisingly already the same (7.43/7.40). Concentration combination of 5-10% showed the same value both upper and bottom layer [6]. It would be the advantage if we used these combinations for sperm sexing, whereas there was no difference pH condition in sexing media. This pH value will give comfort condition for sperm through sexing treatment with albumin columns, because sperm were not pass internal pH changes. It was reported that the sperm movement was significantly influenced by pH of medium. Up and down the external pH would modify internal pH that regulate sperm motility that linked with mitochondria activity. Structure and function of mitochondria clearly effect to sperm motility. The enzyme at mitochondria active at neutral pH. Decreasing of sperm pH will decreasing sperm motility [7].

In order to decreasing motility, therefore the sperm longevity will also decrease. It is clearly understood if the longevity of sexed sperm with combination of BSA concentration 5% and 10% was longest than others.

Moreover, the sperm longevity at BSA combination 6% and 12% were declined. It is because the higher concentration of BSA will have greater viscosity. This condition occurred because higher concentrations would be thicker, so the time needed to flow in the solution was longer. This was in accordance with the statement that the longer the velocity of solution flow time, the greater the value of viscosity [8].

In this research also showed that the sperm longevity in upper layer longer than the bottom layer both in chilled semen and post thawing semen. It is because of the sperm in the bottom layer has experienced movement from the upper layer to the bottom layer, so it has spent more energy than sperm in upper layer. Moreover, it will affect its longevity. In research before It was reported 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 [9]. Moreover, it was reported that 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.

Other researcher reported another factor that causes greater membrane damage. It is the faster movement of sperm in the bottom layer, resulting the level of metabolism is higher, and the reactive oxygen species (ROS) produced is greater than the sperm in the upper layer [10].

The result also showed that the post thawed sexed semen has lower sperm longevity than chilled sexed semen. It because of during 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 [11, 12]. It was reported that semen storage being in liquid or cryopreserved forms is generally associated with a decrease in sperm characteristics, thus a loss in fertility [13, 14]. The major changes following storage are ultrastructural, biochemical and functional and such alterations consequently lead to impaired transport, decline in survival of spermatozoa in the female reproductive tract and reduced fertility [13, 15]. Ram sperm membrane is particularly rich in polyunsaturated fatty acids (PUFAs), making them highly susceptible to cold shock and lipid peroxidation [16]. Impaired cell functions caused by oxidative stress gradually result in decreased sperm viability, morphological and acrosome integrities [17].

Base on the result of the research, it is concluded that the combination of BSA concentration as much 5% in upper layer and 10% in bottom layer resulting longest sperm longevity both in chilled and post thawed sexed sperm.

4. CONCLUSION

The combination of bovine serum albumin (BSA) concentration of 5% and 10% at upper and bottom layer resulting longest sperm longevity both in chilled and post thawed sexed sperm.

AUTHORS' CONTRIBUTIONS

Author contribution in the research consist of main idea, research design and data analysis.

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REFERENCES

[1] R.J. Ericsson, C.N. Langevin and M. Nishino. 1973. Isolation of fractions rich in human Y sperm. Nature (246): 421-424.

[2] W.M.C. Maxwell, G. Meddoa, I.G. Shite (1984). Post thawing survival of motile ram sperm after isolation by layering on protein columns. eriogenol. 21: 601-606.

[3] X. G Zhang, G.J. Yan, J.Y. Hong, Z.Z. Su, G.S. Yang, Q.W. Li, and J.H. Hu. 2015. "Effects of Bovine Serum Albumin on Boar Sperm Quality During Liquid Storage at 17°C." J Reprod. Domest. Anim. 50:263–69. doi: DOI: 10.1111/rda.12481.

[4] N. Solihati, S. Soeparna, S.D. Rasad, and R. Ferlianthi. Proportion and Quality of X-Y Chromosome Bearing Sperm on Diluted Semen after Incubation in Different Time of Etawah Crossbreed Goat. In Procedings of the 7th International Seminar on Tropical Animal Production. Contribution of Livestock Production on Food Sovereignty in Tropical Countries, in September 12-14, 2017, Yogyakarta, Indonesia. P 696-701.

[5] M. Meistrich (1982). Potentials and limitations of physical methods for separations of sperm bearing an X or Y chromosome prospects for sexing Mammalian sperm. Colorado Associated Univ. Press, Boulder, Colorado. Pp. 145-163.

[6] N. Solihati, S.D. Rasad, N. Hilmia, Toha, K. Winangun, Characteristic Several Level of Bovine Serum Albumin (BSA) and Its Combination as Albumin Column for Sperm Sexing, Prosiding Seminar Nasional Teknologi Peternakan dan Veteriner, Bogor Indonesia, 2021, pp. 386–394. DOI: http://dx.doi.org/10.14334/Pros.Semnas.TPV-2020-p.386-394

[7] A Contri, A Gloria, D Robbe, C Valorz, L Wegher, A Carluccio. 2013. Kinematic study on the effect of ph on bull sperm function. J. Anim. Reprod. Sci. 136(4): 252-259.

[8] B.M.E. Jati, A.P. Rizkiana. 2015. Studi penentuan viskositas darah ayam dengan metode aliran fluida di dalam pipa kapiler berbasis hukum poisson. Jurnal Fisika Indonesia. 19(57): 43-47.

[9] N. Solihati, S.D. Rasad, K. Winangun, Toha, A. Yusrina, C. Noviana. Viability of Ram's X-Y Sperm After Sexing with Bovine Serume Albumin at Different Incubation Time. In Proceding of 1st International Conference of Animal Science and Technology (ICAST) 2018, IOP Conference Series: Earth and Environmental Science. Doi:10.1088/1755-1315/247/1/012031

[10] B.T. Storey. 2008. Mammalian Sperm Metabolism: Oxygen and Sugar, Friend and Foe. International Journal Developmental Biology 52: 427-437.

[11] C de F Lucio, D de S.R Angrimani, M.M Brito, and C. I. Vannucchi. Oxidative Stress Challenges during The Sperm Cryopreservation in Dogs. Journal of Veterinary Andrology ISSN 2542-304. 2017. Vol 2 (1): 1-7.

[12] C. Gangwara, A. Saxena, A.Patel, S.P. Singh, S. Yadav, R. Kumara and V. Singh. 2018. Effect of Reduced Glutathione Supplementation on Cryopreservation Induced Sperm Cryoinjuries in Murrah Bull Semen. Animal Reproduction Science 192 (2018): 171–178

[13] S. Salamon and W.M.C. Maxwell. 2000. Storage of Ram Semen. Animal Repro- duction Science, 62, 77-111. https://doi.org/10.1016/S0378-4320(00)00155-X

[14] L. O'Hara, J.P. Hanrahan, L. Richardson, A. Donovan, S. Fair, A.C.O. Evans, and P.Lonergan. 2010. Effect of Storage Duration, Storage Temperature, and Diluent on the Viability and Fertility of Fresh Ram Sperm. Theriogenology, 73, 541-549. https://doi.org/10.1016/j.theriogenology.2009.10.009

[15] W.M.C. Maxwell, and S. Salamon 1993. Liquid Storage of Ram Semen: A Review. Reproduction, Fertility, and Development, 5, 613-638. https://doi.org/10.1071/RD9930613

[16] S.L. Kameni, F. Meutchieye, F. Ngoula. 2021. Liquid Storage of Ram Semen: Associated Damages and Improvement. Open J. of Anim Sci, 2021, 11, 473-500. https://www.scirp.org/journal/ojas

[17] M. Gundogan, D. Yeni, F. Avdatek and A.F. Fidan. 2010. Influence of Sperm Concentration on the Motility, Morphology, Membrane and DNA Integrity along with Oxidative Stress Parameters of Ram Sperm during Liquid Storage. Animal Re- production Science, 122, 200-207. https://doi.org/10.1016/j.anireprosci.2010.08.012