The Effect of Fructose Addition in Semen Extender on Quality of Separation of Garut Ram Sperm in Several Storage Length

(Pengaruh Penambahan Fruktosa dalam Pengencer Sperma terhadap Kualitas Pemisahan Spermatozoa Domba Garut pada Berbagai Lama Penyimpanan)

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Abstract. The objective of the research were to find out the effect of fructose addition in semen extender to the quality of separation Garut ram sperm and their length viability (up to 40% motility) in cool storage. Research design was split plot with four treatments of fructose addition in coconut water extender as a main plot i.e.: 1% (P1); 1.25% (P2); 1.50 (P3) and 1.75% (P4) and the length of sperm viability in cool storage (0; 3rd ; 6 th;9 th;12 th and 15 th hour), as a subplot. Each treatment was repeated five times. Sperm separation was done by albumin column method with 10 % concentration in top layer and 30% in lower layer. Parameters of research were sperm motility and abnormality after separation. Research result showed that addition of 1.75% fructose has the best sperm motility of 57.18± 1.43% and abnormality of 5.76±0.20% in 12 hours cool storage (top layer of albumin column). Sperm motility of 57.62 ± 0.65 % and abnormality of 4.35 ± 0.25% in 12 hours cool storage (lower layer of albumin column). As a conclusion, addition of fructose in coconut water semen extender has positive influenced on sperm quality.

Key Words: fructose, sperm quality, extender, Garut ram

Introduction

Technology of artificial insemination (AI) has efficiently applied to Garut sheep. AI using separation sperm is more efficient due to enhance the livestock population according to the market demand. By administering separation sperm of X and Y in AI technique, the purpose of keeping animals will be achieved. Saili (1991) stated that egg albumin can be used to substitute Bovine Serum Albumen as a method of sperm separation (Seidel, 2007; Gilab *et al*., 2003; D'Alessandro *et al*., 2001))

Semen extender is needed to keep the sperm motility. Coconut water is an alternative semen extender which can be used to provide nutrition and other supporting agent for sperm. Coconut water is also known as a substitution for physiology NaCl in the field. Coconut water contains high protein but low sugar. Therefore it needed to be added by fructose.

Fructose is a kind of sugar which can be easily changed into energy. Fructose addition can be a main source of energy for the sperm. The use of sugar such as fructose, sucrose, glucose, trehalose, EDTA or rafinose in semen extender, could increase sperm motility (Aisen *et al*., 2000; Suwarso, 1999 in Arifiantini, 2006). Sugar in semen extender could keep osmotic pressure, energy source and as a cryoprotectan.

Separation process causes decreasing of sperm quality. Addition of fructose will enhance sperm motility especially along the procedures like albumen penetrated and centrifugation process and the energy sources from nature as a nutrition of spermatozoa could be influenced of length of storage until storage time (Hafez and Hafez, 2000; Garner, 2001).

Research Methods

Material of this reseach was semen of one Garut sheep with 45 Kg weight. Sheep's feeding in this research was 4,5 Kg grass/day and concentrate 0,5 k/day at twice a day. As an extender addition Fructose, Albumen as sperm separation and coconut water as an extender (80% coconut water and 20% egg yolk) were used. Research design was split plot with four treatments of fructose addition in coconut water

extender as a main plot i.e.: 1% (P1); 1.25% (P_2); 1.50 (P_3) and 1.75% (P_4) and the length of sperm viability in cool storage (0; 3rd ; 6 th;9 th;12 th and 15th hour), as a subplot. Each treatment was repeated five times. Sperm separation was done by albumin column method with 10% concentration in top layer and 30% in lower layer. Parameters of research were sperm motility and abnormality after separation.

Results and Discussion

Effect of addition of fructose to sperm motility in several storage length

Top Layer (10% Albumen)

Sperm motility after treatment could be seen in Table 1. Table 1 showed that average highly motility in treatment with Fructose 1.75% with length of storage until 15 hour. Whereas the lower motility was treatment with fructose 1.0%. Based on evaluation in this research, adding 1.75% and 1.50% fructose in coconut water extender with length of storage 12 hour was resulted minimum sperm motility which was suitable for Artificial Insemination Program (AI) (more than 40% sperm motility). Whereas, the lower level of fructose 1.25% and 1.0% in coconut water extender and the motility was decreased faster than other treatments and was influenced length of storage after separation, which the optimal storage not longer than 9 hour, because could be influenced of sperm fertility.

To evaluate the effect of fructose in several storage length coconut water to sperm motility, analysis variety was used. Based on result, there was interaction between level of fructose and length of storage to sperm motility in top layer. That analysis showed, the higher level of fructose in coconut water extender, higher of sperm motility. The analysis was followed with simple test to find out effect of each factor.

Based on simple test on fructose and length of storage, there was interaction between level of fructose and length of storage at top fraction. Data showed that adding fructose 1.75% was influenced the motility compared with 1.5, 1.25 and 1.0% fructose with length of storage from 0 until 15 hour. Increasing level of fructose (1.75%) could be increased sperm motility optimum until 12 hour storage. But declining of the sperm motility from 0 until 15 hour storage was occurred, because of kind of extender, especially composition of the extender which was used in this research. Spermatozoa needs the nutrition for their viability.

Sperm motility after separation lower then without separation before storage at Temperature 5°C, therefore spermatozoa needs more energy for penetration 10% albumen layer appropriate with size of spermatozoa X, whereas bigger then spermatozoa Y. Adding of 1.75% fructose in coconut water extender as a main extender in top fraction (Spermatozoa X) could be produced more ATP *(adenotriphosphate*), and then ATP could be influenced sperm motility after separation, as a main energy of spermatozoa. The metabolism of fructose to energy was occurred faster, because directly change to fructose-6-phosphat (6P), which was used of spermatozoa in their metabolism activity. Therefore, how important fructose as a simple sugar for spermatozoa.

Ax *et al*. (2000) said, temperature of extender was influenced on sperm motility. Therefore, longer length of storage (at Temperature 5°C), lower percentage of sperm motility, based on energy resources in extender. It could be more attention, because the suitability of sperm motility for AI program not lower then 40% (Ax *et al*., , 2000).

Lower Layer (Albumen 30%)

Average motility after treatment fructose 1.0%, 1.25%, 1.50% and 1.75% with length of storage until 15 hour in lower layer could be showed in Table 2. Data showed that increasing level of fructose was influenced on lower layer at every hour evaluation. Average higher motility was in treatment fructose 1.75% until storage 15 hour. Whereas the lower motility was treatment fructose 1.0%. Adding fructose in coconut water extender suppose to be stored not longer then 9 hour, because the limitation of energy resources could be influenced of sperm fertility.

In this research, average sperm motility in treatment 1% fructose after 9 hour storage was 47.90±1.16%, it was lower sperm motility, but this condition could not suitable for AI program.

Based on data on Table 2, to evaluate the influence of each factor, the interaction was analysed with analysis variety. Result of that

analysis showed, there were significant different between two factor (P<0.05). Its means fructose and length of storage factors were influenced to the sperm motility and to find out the interaction between treatments, analysis of simple test was done and the result showed, that level fructose 1.75% was influenced sperm motility higher compare treatment 1.5, 1.25 and 1.0 % fructose with length of storage from 0 until 15 hour.

Increasing level of fructose more than normal in spermatozoa could be energy resources which was used in metabolic process to produce energy ATP, in such away that sperm motility could be increased and the viability of spermatozoa could be longer than normal. Ponglowhapana *et al*. (2004) said, Fructose maintained higher sperm motility.

In the condition under temperature 5°C metabolic activity decline, but spermatozoa cells constancy keep their live in level of capability of survival of the fertility in the certain time (Salisbury and van Demark, 1985). Effect of motility between level of fructose (1.0, 1.25, 1.5, and 1.75 %) in every time caused of fructose as a energy source and nutrition for the spermatozoa.

Increasing of fructose 1.75% was influenced higher motility and longer of length of storage both of two layer, top fraction (spermatozoa X) or below fraction (spermatozoa Y). Increase the time could be longer of viability and sperm motility. Gilab *et al*. (2003) and Marti *et al.* (2003) said, that sperm motility needs energy, therefore the spermatozoa needs more energy sources after separation compare with spermatozoa without separation. Furthermore, treatment of fructose could be decrease of damaging of sperm permeability. The permeability close connected with nutrition transportation which was important for metabolic cell to produce of energy. Decreasing of damaging of membrane permeability of sperm, nutrition consumption could not be blocked and the viability cell spermatozoa could be longer. This condition supported by Hidayaturrahman (2007), that permeability of spermatozoa membrane close connected with motility and viability of sperm.

Decreasing of sperm motility after separation and storage from 0 until 15, because many process was occurred by the spermatozoa, which was needed more extra energy. By storage in temperature 5°C, decreasing of sperm motility was occurred, because in the lower temperature metabolic of cell spermatozoa decline, continued of declining of sperm motility.

In an aerobic condition (T 5°C) between storage from 0–15 hour, there was no enough oxygen for metabolic fructose. This fructolysis activity could be maintained sperm motility during storage. Therefore, increasing fructose 1.75% could be maintained sperm motility after separation in below fraction longer than other level of fructose. Decreasing of sperm quality after separation during storage was occurred, because many process by spermatozoa was done and storage in temperature 5°C.

Table 1. Average sperm motility in top layer (Albumen 10%)

Treatment	Length of Storage (Hour)					
	0	3	6	9	12	15
	.....%					
1.0%	72.00±2.18	62.14±1.67	54.52±2.27	45.07±1.77	30.43±2.40	14.05±1.50
1.25%	76.57±1.33	70.34±0.94	60.77±1.68	50.70±1.11	39.03±1.15	18.52±1.48
1.50%	79.32±1.65	75.36±1.75	71.01±1.72	63.10±1.31	42.46±1.28	34.02±0.96
1.75%	83.18±1.89	79.10±1.67	73.75±1.11	66.61±1.11	57.18±1.43	37.89±0.54

Table 2. Average sperm motility in lower layer (Albumen 30%)

Treatment	Length of Storage (Hour)					
	0	3	6	9	12	15
	.....%					
1.00%	75.48±1.77	66.08±1.66	58.11±1.50	47.90±1.16	32.94±2.28	17.55±1.31
1.25%	79.49±1.61	74.10±2.20	63.90±2.00	55.13±2.00	43.42±2.22	21.61±2.62
1.50%	82.76±1.67	76.37±1.27	71.62±1.31	65.86±1.87	52.27±1.45	35.41±1.70
1.75%	86.08±1.91	80.22±1.38	75.56±1.10	67.53±0.65	57.62±0.92	38.95±0.42

Others, decreasing of motility occurred, because increasing amount of sperm mortality, its could be toxic to other spermatozoa. In that condition, fructose also as a extra cellular cryoprotectant agent, which was plasma membrane spermatozoa protected of toxicity during storage (Johnson *et al*., 1999). Salamon and Maxwell (1995) said, that sperm viability was decreased during storage at temperature of 5°C in many kind of extender, which have nutrition in that extender for spermatozoa.

Increasing of fructose 1.75% could be extended sperm viability and maintained the motility. Toelihere (1993) explained, that fructose as carbohydrate derived which could be energy sources to support motility and viability of sperm. Energy resources was enough, could be maintained the viability of sperm longer than lower level of fructose.

Effect of addition of fructose to abnormality of sperm in several storage length

Top Layer (Albumen 10%)

Abnormality of sperm could be used as a one of condition to semen evaluation for AI program. Data of sperm abnormality after treatment fructose and storage until 15 hour showed in Table 3. Increasing of abnormality of sperm was occurred compare with chilled semen without separation. In this research, average higher number of sperm abnormality was in treatment 1.0% fructose with length of storage 12 hour (6.26±0.32) compared with 1.75% fructose (5.80±0.47) with the same length of storage (12 hour).

Separation or sexing process could be lose of energy by spermatozoa and the membrane plasm could be thickness. Increasing of fructose in the extender could be protected the sperm. Fructose as a cryopotectant agent extra and intra cellular. Klinc and Rath (2006) suggested, that sugar with big molecule weight could be played role as extra cellular cryprotectant, whereas cryoprotectant agent intracellular have smaller molecular weight. In this condition, fructose penetrate into intracellular cryoprotectant, whereas fructose could penetrated cell spermatozoa wall into cell (Paulenz *et al*., 2009).

Sperm storage at 15 hour could be produced abnormality of sperm less than 20%, this condition was still in normal using in AI program. Toelihere (1993) suggest, that semen with abnormality sperm less than 20% are still normal for AI program. Ax *et al*. (2000) said, that percentage of abnormality of sperm not more than 30-35%. Variety analysis between two factors to sperm abnormality showed, that there was no interaction, but there was influenced fructose factor on sperm abnormality, and length of storage on sperm abnormality. There was different effect of fructose on abnormality in treatment 1.0%, 1.25% and 1.50% compared with 1.75% fructose, and length of storage 15, 12 and 9 hour was not significant different ($P>0.05$), but significant different ($P<0.05$) higher compare with 0, 3 and 6 hour storage. Longer length of storage could be influenced on motility and abnormality of sperm. This indicated, that longer length of storage could be increased of abnormality morphology of sperm. Abnormality sperm in this research was secondary abnormality like break between head and tail of sperm, lack of tail or abnormality of size of sperm.

Effect of morphological abnormality which was increased, because of loss of the energy and during of centrifugation (Rijsselaere *et al*., 2002). High speed of centrifugation could be influenced morphology of sperm after separation, especially during penetrating of sperm in albumen layer in both 10 or 30%. Suh *et al*. (2005) hypothesized that lowering the sorting pressure could reduce sperm damage.

Lower Layer (Albumen 30%)

Table 4 showed, that average abnormality from 0 until 15 hour storage tend to increased. The abnormality was positive correlation with motility, if abnormality high, the motility could be decline. Length of storage until 15 hour was a higher sperm abnormality in treatment 1.0% fructose, but the lower was treatment fructose 1.75%. Higher level of fructose, lower percentage of sperm abnormality, but until storage 15 hour, semen was normal for AI program (less than 20%).

Secondary Abnormality was found in this research, like lack of tail, break between tail and head, abnormality of tail. This condition was occurred, because osmotic pressure during storage, which was lower temperature during storage than room temperature. Salamon and

Maxwell (1995) explained, that change of room temperature could be change morphology of sperm.

Variety analysis showed, that there was interaction between fructose and length of storage, but each factor was significant influenced on sperm abnormality in lower layer. By Multiple distance Duncan Test showed that between treatment 1.0%, 1.25% and 1.50% were not significant different (P>0.05), but significant different (P<0.05) compared with fructose 1.75%. Its means, increasing of level of fructose, lower sperm abnormality, or conversely. This was indicated, that adding of fructose in coconut water extender could be used by spermatozoa for energy resources to produce ATP as a energy source and cryoprotectant agent. High level of fructose could be protected spermatozoa inside cell, because fructose could be penetrated into cell of sperm and make coordinating with fibril micro in middle of tail of sperm to maintain the morphology of sperm. The type of sugar significantly affected motility, viability and acrosome integrity during equilibration and freezing (Yildiz *et al*., 2000).

Percentage of sperm abnormality after storage 15, 12 and 9 hour higher than 0, 3 and 6 hour storage. The abnormality sperm after storage between 15, 12 and 9 hour was not significant different (P>0.05), whereas storage 0, 3 and 6 hour was significant (P<0.05) influenced on sperm abnormality after separation. This data indicated, that longer length of storage could be influenced on level of abnormality of sperm. This occurred, because the fibril in tail was not worked, so that the morphology of sperm become abnormal.

Conclusions

Different level of Fructose addition contained in coconut water extender and the length of keeping cool storage are significantly affect the quality of separation Garut Ram sperm.

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Table 3. Average Sperm Abnormality In Top Layer (Albumen 10%)

Treatment	Length of Storage (Hour)					
	0	3	6	9	12	15
	.....%.....					
1.00%	5.16±0.17	5.48±0.12	5.65±0.16	6.00±0.18	6.08±0.36	6.26±0.32
1.25%	5.24±0.09	5.28±0.32	5.72±0.40	5.87±0.35	5.98±0.40	6.18±0.31
1.50%	5.10±0.16	5.33±0.47	5.68±0.45	5.73±0.33	5.85±0.39	6.04±0.30
1.75%	5.30±0.19	5.34±0.44	5.35±0.20	5.48±0.43	5.79±0.54	5.80±0.47

Table 4. Average Sperm Abnormality in Lower Layer (Albumen 30%)

Treatment	Length of Storage (Hour)					
	0	3	6	9	12	15
	.....%.....					
1.00%	5.17±0.64	5.56±0.54	6.15±0,42	5.99±0.58	6.10±0.36	6.46±0.46
1.25%	4.99±0.53	4.93±0.56	5.52±0,48	6.00±0.32	6.46±0.20	6.43±0.17
1.50%	4.76±0.42	4.85±0.46	4.98±0,36	5.61±0.26	5.74±0.41	6.22±0.21
1.75%	4.52±0.35	4.72±0.42	4.95±0,38	5.22±0.39	5.88±0.31	5.94±0.36

References

Arifiantini RI. 2006. Preservasi Semen dengan Modifikasi Pengencer dan Krioprotektan untuk Inseminasi Buatan pada Kuda. Disertasi Program Pascasarjana Institut Pertanian Bogor. Bogor.

Ax RL, M Dally, BA Didion, RW Lenz, CC Love, DD Varner, B Hafez, and ME Bellin. 2000. Semen Evaluation. In: Reproduction In Farm Animals. ESE Hafez and B Hafez (Ed). 7th Ed. Baltimore : Lippincot Williams & Wilkins. Pp. 376.

D'Alessandro AG, G Martemucci, MA Colonnac and A Bellittic. 2001. Post-thaw survival of ram spermatozoa and fertility after insemination as affected by prefreezing sperm concentration and extender composition. *Theriogenology* 55: 1159-1170.

Garner DL. 2001. Sex-Sorting Mammalian Sperm: Concept to Application in Animals. *J. Andrology* 22: 4.

Gilab J, N Lundeheim, L Söderquist and HR Martin. 2003. Influence of extender, temperature, and addition of glycerol on post-thaw sperm parameters in ram semen. *Theriogenology* 59(5-6):1241-1255

Hafez ESE and B Hafez. 2000. X and Y Chromosome-Bearintg Spermatozoa. In: Reproduction in Farm Animals ESE Hafez and B Hafez (Ed). 7th Edition. Baltimore : Lippincot Williams & Wilkins. Pp. 390.

Hidayaturrahman. 2007. Waktu motilitas dan Viabilitas Spermatozoa Ikan Mas Pada Beberapa Konsentrasi Larutan Fruktosa. *Bioscientiae* 4(1): 9-18.

Johnson LA, GR Welch and W Rens. 1999. The Beltsville Sperm Sexing Technology: High-Speed Sperm Sorting Gives Improved Sperm Output for In Vitro Fertilization and AI. *J. Anim. Sci.* 77: 213-220.

Klinc P and D Rath. 2006. Application of flowcytometrically sexed spermatozoa in different farm animal species: a review**.** *Archiv Tierzucht, Dummerstorf* 49(1): 41-54.

Marti JI, E Martí, JA Cebrián-Pérez and T Muiño-Blanco. 2003. Survival rate and antioxidant enzyme activity of ram spermatozoa after dilution with different extenders or selection by a dextran swim-up procedure. *Theriogenology* 60 : 1025-1037.

Paulenz H, L Söderquist, R Perez and KA Berg. 2009. Effect of different extenders and storage temperatures on sperm viability of liquid ram semen. *Theriogenology* 57(2):823-836.

Ponglowhapana S, BE Gustavssonb and CL Forsberg. 2004. Influence of glucose and fructose in the extender during long-term storage of chilled canine semen. *Theriogenology* 62: 1498-1517.

Rijsselaere T, AV Soom, D Maes and A de Kruif. 2002. Effect of centrifugation on in vitro survival of fresh diluted canine spermatozoa. *Theriogenology* 57:1669-1681.

Saili T. 1999. Efektivitas Penggunaan Albumen Sebagai Medium Separasi Dalam Upaya Mengubah Rasio Alamiah Spermatozoa Pembawa Kromosom X dan Y pada Sapi. Tesis. PPS IPB, Bogor.

Salamon S and WMC Maxwell. 1995. Frozen Storage of Ram Semen I. Processing, Freezing, Thawing, and Fertility After Cervical Insemination. *Anim. Rep. Sci.* 37:185-249.

Salisbury GW and NLVan Demark. 1985. Fisiologi Reproduksi dan Inseminasi Buatan Pada Ternak. Terjemahan: Djanuar. Gajah Mada University Press. Yogyakarta. Hal. 201-343.

Seidel GE Jr. 2007. Overview of sexing sperm. *Theriogenology* 68:443-446.

Suh TK, JL Schenkb and GE Seidel Jr. 2005. High pressure flow cytometric sorting damages sperm. *Theriogenology* 64:1035-1048.

Toelihere MR. 1993. Inseminasi Buatan Pada Ternak. Penerbit Angkasa. Bandung.

Yildiz C, A Kaya, M Aksoy and T Tekeli. 2000. Influence of Sugar Supplementation of the Extender on Motility, Viability, and Acrosomal Integrity of Dog Spermatozoa During Freezing. *Theriogenology* 54:579-585.