Fertility of frozen-thawed ejaculated and epididymal sperm of Garut ram

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Introduction

Cauda epididymal sperm could be used as an alternative of gamete source for application in reproductive technology, because the sperm is motile and have ability for fertilizing the oocyte (Axner et al. 1999; Hafez and Hafez 2000). This sperm could be collected for life or death animal, then it might be processed for chilling or freeing. The purpose of this research was to examine and compare the quality of sperm and fertility between frozen-thawed ejaculated (EJS) and frozen-thawed epididymal (EPS) sperm followed by artificial insemination (Al).

Materials and Methods

Semen was collected from three Garut rams using an artificial vagina once a week. Epididymal sperm was collected from cauda epididymis of slaughtered-ram Semen was diluted with modified Tris extender containing 5% glycerol and 20% egg yolk. The modified Tris extender is containing 3.32 g Tris (hydroxymethyl) aminomethane, 1.86 g citric acid-monohydrate, 1.37 g D(-) fructose, 2.16 g lactose-monohydrate, 0.05 g glutathione, 1000 IU/ml penicillin, and 1000 µg/ml streptomycin in 100 ml distilled water. Semen was loaded in 0.25 ml mini straw with the final concentration of 200 million motile sperm. Semen was equilibrated at 5 °C for three hours. The straws were placed for 15 minutes in liquid nitrogen vapor. 10 cm above the level of liquid nitrogen. The straws were then plunged into liquid nitrogen and stored at –196 °C. Straws were thawed in a 37 °C water bath for 30 seconds prior to analysis. Quality of processed-semen including percentages of motile sperm, live sperm, and intact plasma membrane (IPM; Revell and Mrode 1994) were evaluated after diluting, equilibrating and thawing, respectively. Before insemination, estrus of ewes were synchronized uring intravaginal administration of CIDR-G® for ~ 13 days. Cervical insemination war carried out 53 hours after withdrawal of CIDR-G® and repeated seven hours later with one dose, respectively. Pregnancy was determined with ultrasonography (USG) 83 days after insemination.

Results and Discussion

Results of this research showed that sperm quality of EJS is better than EPS. Mean percentage of motile sperm after diluting for EJS (76.67%) was significantly ($P<0.05$) higher than EPS (70.83%). There was no significantly difference between EJS and EPS for mean percentages of live sperm and IPM after diluting. After equilibrating, mean percentages of motile sperm, live sperm, and IPM for EJS (70.00%, 77.00%, and 74.44%) were significantly ($P<0.05$) higher than EPS (58.33%, 70.50%, and 68.33%). Mean percentages of post thawing motile sperm, live sperm, and IPM for EJS (52.78%, 58.78%, and 56.22%) were significantly ($P<0.05$) higher than EPS (45.00%, 54.50%, and 48.83%) (Table 1). Pregnancy and lambing rate for EJS (58.33% and 58.33%) was significantly ($P<0.05$) higher than EPS (44.44% and 33.33%) (Table 2).

Table 1. Percentages of motile sperm, live sperm, and IPM of ejaculated and cauda epididymal sperm of Garut ram

Parameters of semen quality	Phase of semen Processing	Treatment	
		EJS	EPS
Motile sperm (%)	Diluting	76.67 ± 2.36[b]	70.83 ± 1.86'
	Equilibrating	70.00 ± 4.08[b]	58.33 ± 2.36[a]
	Thawing	52.78 ± 2.48[b]	45.00 ± 4.08[a]
Live sperm (%)			
	Diluting	82.89 ± 3.35'	82.83 ± 1.57"
	Equilibrating	77.00 ± 2.98[b]	70.50 ± 2.87[a]
	Thawing	58.78 ± 1.47[b]	54.50 ± 2.14[a]
IPM (%)			
	Diluting	83.55 ± 1.83[a]	81.33 ± 1.10[a]
	Equilibrating	74.44 ± 23 1[b]	68.33 ± 1.50[a]
	Thawing	56.22 ± 1.81[b]	48.83 ± 2.19[a]

[a,b] superscrips in a row indicating significant difference (P<0.05), n = 6
EJS= ejaculated sperm, EPS = epididymal sperm, IPM = intact plasma membrane.

Table 2. Pregnancy and lambing rate of frozen-thawed sperm by cervical artificial insemination

Frozen-thawed	Pregnancy rate (%)	Lambing rate (%)	Number of lamb		
			Single	Twin	Total
EJS	7/12 (58.33)[b]	7/12 (58.33)[b]	5	2	9
EPS	4/9 (44.44)[a]	3/9 (33.33)'	3	_	3

[e,b] superscrips in a column indicating significant difference (P<0.05)
EJS = ejaculated sperm, EPS = epididymal sperm.

Quality of ejaculated sperm is better than epididymal sperm may be caused by its have a seminal plasma. The seminal plasma is containing several compounds such as lipoprotein to protection of sperm plasma membrane (Gilbert 1988; Johnson and Everitt 1995; Nolan and Hammerstedt 1997) and stimulating sperm motility (Squires et al. 2000). The motility of epididymal sperm treated with seminal plasma was similar to that of ejaculated sperm (Squires et al. 2000). Therefore, fertility of frozen-thawed ejaculated sperm is higher than frozen-thawed epididymal sperm. Pregnancy and lambing rate for frozen-thawed ejaculated sperm (EJS) of this research was similar with reported by McPhie et al. (2000). The same goes for frozen-thawed epididymal sperm (EPS) was similar with reported by Bravo et al. (2000) at llamas and alpacas.

In conclusion, quality of frozen-thawed ejaculated sperm is better than frozen-thawed cauda epididymal sperm. Frozen-thawed ejaculated and caude epididymal sperm of Garut ram are suitable for use in artificial insemination by cervical method.

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