

# THE EFFECT OF CAFFEINE EGG YOLK TRIS SEMEN EXTENDER TO THE QUALITY OF SEPARATION OF GARUT RAM SPERM

Soeparna Ibrahim, Tita Damayanti Lestari, Lies Nurfaidah

*Laboratory of Animal Reproduction, Faculty of Animal Husbandry,  
Universitas Padjadjaran-Bandung, West Java, Indonesia  
e-mail: titadlestari@unpad.ac.id*

## Abstract

*Study on the effect of caffeine within egg yolk Tris semen extender to the quality of separation of Garut ram sperm has been conducted. The aims of the study were to know the effect of caffeine in egg yolk Tris extender on motility and velocity of the sperm after separation and to find out the optimal dose of caffeine for both parameters. This study used Completely Randomized Design with four treatments on different dose of caffeine (0 mM; 2 mM; 4 mM and 6 mM) and each treatment was repeated five times. Results showed that addition of caffeine into egg yolk Tris extender was significantly affect sperm motility ( $P < 0.01$ ) of Garut ram sperm after separation and the best dose of caffeine was 4 mM to be added into Egg Yolk Tris semen extender to obtain the highest sperm motility and velocity.*

**Key words:** Sperm, Garut ram, sperm separation, egg yolk Tris, caffeine

## INTRODUCTION

Garut sheep is a potential meat source for consumption. Therefore its reproduction should be foster by biotechnology. One of applied technology is artificial insemination which requires chilled or frozen semen. As technology developed, sex of offspring can be determinate by using separated sperm Y and X.

Sperm separation is done in order to get sperm with special chromosome to support the purpose of rearing the animal. Male offspring in livestock is proposed to be reared as feedlot, because male animal has faster weight gain and it is economically profitable. Female offspring is proposed to be kept for dairy and reproduction achievement. On the other hand, separation process brings a risk of decreasing sperm quality especially on sperm motility and velocity, and finally decreasing sperm viability to fertilize ovum. To get over the problem, addition of caffeine in the semen extender is probably become a solution, since caffeine is a substance which can stimulate sperm progressivity.

Concerning the problem above, a research should be done. This research is conducted to find out what dose of caffeine

which can increase the quality of sperm after separation.

## MATERIAL AND METHOD

This research used ram semen which was diluted by BO solution. BO media was made by mixing 76 ml part A solution and 24 ml part B solution added by sodium pyruvate and antibiotic (penicillin 100 IU/ml media and streptomycin 0.05 mg/ml media). Ram semen was collected using artificial vagina. Then evaluated macroscopically and microscopically.

Albumen used in this research derived from broiler chicken egg. Thin albumen was sucked by Pasteur pipette. Separated coulomb was 10% albumen in BO media as top layer and 30% albumen in BO media as lower layer, placed in a glass tube with comparison of 1:1.

Semen then diluted by BO media with concentration of 200 million per ml, and 1 ml semen then poured into top layer of albumen. Put in the water bath for one hour. Top layer was assumed contained X sperm and lower layer was assumed as Y sperm. Separation semen then diluted by egg yolk Tris extender

and added with 0 mM, 2 mM, 4 mM. and 6 mM. caffeine. Parameters observed were sperm motility and velocity.

This study used Completely Randomized Design with four treatments on different dose of caffeine (0 mM; 2 mM; 4 mM and 6 mM) and each treatment was repeated five times. Data collected was analyzed by Duncan Test.

**RESULTS AND DISCUSSIONS**

Effect of caffeine on motility of Garut ram sperm is showed in Table 1. Table 1 shows that adding of caffeine in separation semen is highly significant influenced (P<0.01) to the sperm motility. Duncan test results show the addition of 4mM caffeine gives the highest motility compare to other doses, on top (63.57%) and low layer (65.90%) of albumen coulomb. The role of phosphodiesterase (PDE) enzyme in caffeine

is probably resulted high cAMP and as a consequence produce high energy for the sperm.

The role of caffeine in increasing level of cAMP with A-kinase enzyme is in order to increase phosphorilase activity. Then the activity of reaction from glycogen to become glucose is done by glycogen phosphorilase enzyme. Concentration of glucose as energy source increases, and as a consequence increasing the sperm metabolism and sperm motility (Soedarsono, et al, 1995 and Lieberman, 1988).

Sperm motility in treatment of 6 mM caffeine on top layer of coulomb albumen is 58.57% and low layer is 60.07% and are smaller than motility of 4 mM caffeine addition. It is probably because of imbalance solution resulted from higher caffeine concentration in the semen extender and cased of toxicity (Richie, 1987 in Schunack, dkk, 1990).

Table 1. Duncan Test Result on Garut Ram Sperm Motility after Caffeine Addition

Caffeine Dose (mM)	Motility Average (%)		Significance			
	Top Layer	Low Layer	Top Layer (10%)		Low Layer (30%)	
			<b>0.05</b>	<b>0.01</b>	<b>0.05</b>	<b>0.01</b>
4 mM	63.57	65.80	a	a	a	a
6 mM	58.57	60.06	b	b	b	b
2 mM	54.59	57.23	c	c	c	c
0 mM	46.59	48.40	d	d	d	d

Remark: Different letters to the coulomb direction show highly significant different.

Effect of caffeine on sperm velocity is showed in Table 2. Table 2 shows that sperm velocity in treatment of 4 mM is the highest on top layer (62.37), and low layer (65.90%)

compare to other treatments. High velocity of sperm is probably because of the role of inhibitor PDE.

Table 2. Average of Garut Ram Velocity Sperm After Caffeine Addition

Treatment	Sperm Velocity Average	
	Top layer of Albumin Coulomb (X Sperm in μm/second)	Low Layer of Albumin Coulomb (Y Sperm in μm/second)
P <sub>0</sub> (0 mM of caffeine)	47.20	48.40
P <sub>2</sub> (2 mM of caffeine)	55.99	57.23
P <sub>4</sub> (4 mM of caffeine)	62.37**	65.90**
P <sub>6</sub> (6 mM of caffeine)	58.62	60.06
Average	56.04	57.89

The highest sperm velocity value in caffeine of 4 mM dose, is due to existence of PDE inhibitor work. Glicolysis and lipolysis could be increased by the addition of caffeine (Schunack, et al, 1990). This metabolic work

is to inhibit phosphorilase enzyme which is activated hydrolysis adenosine 3-5 monophosphate (cAMP) to become adenosine mono phosphate (AMP).

Elevated cAMP will inhibit dispersing ADP to become AMP, on the other hand, glycolysis and lipolysis within sperm mitochondrial also increase. This means more providing energy sources that could be utilized by velocity sperm.

On the other side sperm velocity value in treatment 6 mM both of top layer of albumin coulomb (58,62  $\mu\text{m}/\text{second}$ ) and lower layer (58, 29  $\mu\text{m}/\text{second}$ ) are lower than on treatment of 4 mM of caffeine, but I is still better than no caffeine extender. This fact is probably because of extender imbalance caused by concentration of caffeine and maybe because of the toxicity of caffeine itself.

### CONCLUSIONS

1. Addition of caffeine in chilled semen post separation can increase ( $P < 0,01$ ) Garut ram sperm motility and velocity.

2. The optimum dose of caffeine is 4 mM to be added into Egg Yolk Tris semen extender.

### REFERENCES

- [1] Afiati, 2004. Proporsi dan Karakteristik Spermatozoa X dan Y Hasil Separasi Kolom Albumen. Jurnal Media Peternakan. Vol.28 No.1 Hal 16-20.
- [2] Bearden, H.J.; J.W. Fuquay and S.T. Willard. 2004. *Applied Animal Reproduction*, Sixth Edition. Prentice Hall-Upper Saddle River. New Jersey.
- [3] Lieberman, S.J.; W.Wasco; J.MacLeod; P. satir and G.A.Orr. 1988. Immunogold localization of regulatory submit of a type II c-AMP-dependent protein kinase tightly associated with mammalian sperm flagella. Departments of anatomy and Structural Biology and Molecular Pharmacology. Albert Einstein College of Medicine. New York. P 1809 – 1816.
- [4] Schunack; W.K. Mayer dan Haake. 1990. Senyawa obat. Buku panduan pelajaran kimia farmasi. Edisis ke 2. gajah Mada University Press. 235 – 367.
- [5] Soedarsono, S.; Soelaeman; Syarifudin dan Soebroto. 1995. Pengaruh penambahan kafein pentoksilin dan papaverin dalam usaha meningkatkan jumlah spermatozoa motil pada swim up untuk inseminasi. MAI,11 : 31 – 42.