The Effect of Caffeine Supplementation in Egg-Yolk Tris Extender on Motility and Velocity of Garut Sheep Sperm Separation

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Abstract. The aims of this research were to know the effect of caffeine in Tris egg yolk extender on motility and velocity of Garut Ram sperm after separation and to find out the optimum dosage of caffeine for both parameters. This research used experimental design with Completely Randomized Design (CRD) with four treatments on different dosage (0 mM, 2 mM, 4 mM and 6 mM), each treatment is repeated five times. The result showed that the supplemtation of caffeine significantly influence sperm motility of Garut Ram sperm after separation. The highest sperm motility was on dosage 4 mM that was 63.57% on top fraction and 65.90% on below fraction. The highest sperm velocity was on dosage 4 mM that was 62.37 µm/second on top fraction and 64.24 µm/second on below fraction and significantly influence sperm velocity. The conclusion, 4 mM dosage of caffeine was the optimum dosage to reach the highest motility and velocity of Garut Ram sperm both on top and below fractions.

Key Words*:* spermatozoa, Garut ram, sperm separation, caffeine

Introduction

The objectives of the optimalization of Artificial Insemination program was fattening and the male livestock develop faster then female. AI technique using sperm separation of X and Y will be successful for that program (Gordon, 2005).

The problem of sperm separation was lower of sperm quality after separation, like ultrastructure and biochemist damage of sperm. This might be caused decrease of motility and velocity of sperm and finally the ability of sperm to penetration of oocytes during the fertilization process could be decrease.

One of the way to increase of sperm quality including motility and velocity of sperm were supplement addition, which was stimulate the motility of sperm. The supplement which was used called caffeine (1,3,7 trimetilxantine). Many research was found, that Caffeine was reised in Vitro fertilization ability by livestock animal or human, because activity of caffeine could be increase fertilization capability of sperm by increasing of cAMP intracellular concentration, it makes the ability of motility and velocity of sperm to penetration of the oocytes would better. There are many efforts in order to substitute egg yolk-based extenders by other synthetic semen diluents, for example AndroMed, Biociphos Plus (Bielanski, 1997), Bioexcell® (Gill, 2003) - for bovine, ram and goat sperm or EquiPro-defined milk protein extender – for stallion sperm (Pagl et al., 2006). To minimize the damages to the sperm during a long-term storage a wide variety of substances such as glutathione, caffeine, cysteine, taurine, hypotaurine, bovine serum albumine, trehalose or hyaluronan have been tested.

Supplementation of caffeine was increased the ability of sperm fertility bay human or livestock animal, but the research about the effect of caffeine addition on separation of sperm not yet publish. The addition of glutathione or caffeine to EquiPro commercial diluents provided higher ewe´s pregnancy rate in comparison to the egg yolk-based diluent. Similarly, Fukui et al. (2007) demonstrated that the use of the semen extender without egg yolk resulted in the fertility rate comparable to egg yolk contained extender (63 and 67% lambing

rates for BSA- and egg yolk-contained extender, respectively). The recent experiments using a commercial synthetic extender–AndroMed (Fukui et al., 2008), demonstrated that an egg yolk-contained semen extender can be replaced with the synthetic extender EquiPro for the insemination of ewes without reducing fertility. Glutathione showed a positive effect on ram semen. It can be used as an additive to semen extender to enhance pregnancy rate of ewes after artificial insemination.

Aim of this research were to know the effect of caffeine in Tris egg yolk extender on motility and velocity of Garut Ram sperm after separation and to find out the optimum dosage of caffeine for both parameters.

Sperm separation with albumen column (bovine serum albumin or human serum albumin) method was done and it was a feasibility method (Hafez and Hafez, 2000). The principle of sperm X and Y separataion based on velocity of sperm motility and the dimension of the spermatozoa Y was smaller than spermatozoa X and the dimension closed connection with DNA contents. It makes the sperm Y could be moved and penetrated the albumin column faster than sperm X.

Bovine serum albumin could be changed with egg albumin (Saili, 1999) and for the maintain the quality of sperm could be used supplement to improve the sperm motility. Aim of the supplement as chemical component sources during sperm maturity as well as morphological and physiological component until fertilization process (Lamming, 1990). One of the supplement was called Caffeine ($C_8H_{10}N_4O_2$ or 1,3,7-Trimethyl xanthine) (Garbers et al., 1973).

Materials and Methods

The research was used experimental method with completely random design and consist of four dosage of caffeine as the treatment (0 mM, 2 mM, 4 mM and 6 mM). every treatment was five times replication. Sperm separation was used egg albumin which was diluted in Bracket and Oliphant's medium (BO medium) until 200 million per ml concentration. Put 1 ml semen in tube including 10% albumin in top fraction and 30% in below fraction. Kept in waterbath until 1 hour. Top fraction as a spermatozoa X and below fraction as a sperm Y was separated in other tube.

Parameter of the research were motility and velocity of sperm. For the velocity was calculated with formula :

$$v = \frac{s}{t}$$

v = sperm velocity (μm/second)
s = travelled distance of sperm (μm)
t = time (second)

Results and Discussion

Effect of Caffeine on Motility of Sperm Separation

Higher average of motility was addition of 4 mM caffeine in top and below fractions (63.57 and 65.09%) (Table 1). This was occurred, because of phosphodiestrease enzyme activities in caffeine which was produced higher cAMP, and it makes the motility better than other treatment in top fraction P_0 (46.59%), P_2 (54.59%), P_6 (58.57%), and below fraction K_0 (48.40%), P_2 (57.23%), P_6 (60.06%).

The mechanism of caffeine was to increased of cAMP levels with enzyme A-Kinase, increasing of phosphorilase activity and activated of phosphorilase kinase and the glycogen phosphorilase enzyme to glucose. Amount of the glucose as a energy sources and metabolism of the sperm were increased. Finally increased the sperm motility (Sudarsono et al., 1995; Lieberman, 1988), because Caffeine is a heterocyclic compound that has been reported to promote hyperactivation in bull and human spermatozoa (Ho and Suarez, 2001; Marques and Suarez, 2004)

Table 1. Average of motility sperm separation after supplementation of caffeine

Treatment	Average of Motility	
	Top fraction (%) (Spermatozoa X)	Below fraction (%) (Spermatozoa Y)
P_0 (Kafein 0 mM)	46,.19	48.40
P_2 (Kafein 2 mM)	54.59	57.23
P_4 (Kafein 4 mM)	63.57	65.90
P_6 (Kafein 6 mM)	58.57	60.06
Average	55.83	57.89

and to enhance sperm motility and improve fertilization (Ho and Suarez, 2001; Pavlok et al., 2001; Marquez and Suarez, 2004).

Sperm motility with addition of 6 mM caffeine in both of the fraction (Top fractions was 58.57 and below fraction was 60.06%) were decreased compared with 4 mM dosage of caffeine (63.57 in top and 65.90% in below fractions). It were occurred, because unbalanced of extender while higher of caffeine level and influence of caffeine toxicities. Higher of certain substance in extender could be decreased of another substance and disturbed sperm metabolism (Salisbury dan VanDemark, 1985). Caffeine have Toxicity effect on nerven system and blood circulation (Richie, 1987 in Schunack, 1990).

Effect of Caffeine on Velocity of Sperm Separation

Average of sperm velocity of Garut Ram spermatozoa cross Neubauer chamber was 56.04 μm/sec in top fraction and 56.81 μm/sec in below fraction. The higher velocity was in 4 mM dosage of caffeine treatment (62.37 μm/sec in top fraction and 65.90 μm/sec in below fraction) and the lower velocity was 0 mM caffeine doses (47.20 μm/sec in top and 48.40 μm/sec in below fraction) (Table 2).

Higher sperm velocity was occurred in treatment P_4 (4 mM dosage of caffeine), the spermatozoa could be crossed the Neubauer Chamber in 62.37 μm/sec in top fraction and 65.90 μm/sec in below fraction, compared with treatment P_0 (Kafein 0 mM).

Result of the significant mean differences of Duncan Test shown, that supplementation of caffeine on chilled semen of Garut Ram was significantly influence ($P<0.01$) on sperm velocity. Higher average of the velocity was 4 mM dosage of caffeine in top fraction (62.37 μm/sec) and below fraction (64.24 μm/sec) (Table 3).

Tabel 2. Average of sperm velocity after supplementation of caffeine

Treatment	Average of sperm velocity (μm/sec)	
	Top fraction (Spermatozoa X)	Below fraction (Spermatozoa Y)
P_0 (Kafein 0 mM)	47.20	48.40
P_2 (Kafein 2 mM)	55.99	57.23
P_4 (Kafein 4 mM)	62.37	65.90
P_6 (Kafein 6 mM)	58.62	60.06
Average	56.04	57.89

Table 3. Sperm velocity after supplementation of caffeine (Duncan test)

Dosage of caffeine (mM)	Average sperm velocity (μm/sec)		Range			
	Top Fraction	Below Fraction	Top fraction (10%)		Below fraction (30%)	
			0.05	0.01	0.05	0.01
4 mM	62.37	64.24	a	a	a	a
6 mM	58.62	58.29	b	b	b	b
2 mM	55.99	56.44	c	c	c	c
0 mM	47.20	48.60	d	d	d	d

Values bearing different superscript at the same column differ significantly (P<0.05).

The higher of the velocity of that treatment (4 mM dosage of caffeine) was done because of inhibitor effect of *phosphodiestrease enzyme (PDE).* Schunack et al. (1990) said, glycolysis and lypolysis could be increased by supplementation of caffeine. Metabolic effect of caffeine could be blocked the PDE. PDE as enzyme which were hydrolysis activated of adenosine 3-5 (cAMP) to adenosine monophosphat. Increase of cAMP could be inhibited of the metabolism to AMP, and caused of increasing the cAMP level in cell and rate of sperm metabolism, especially increased of glycolysis and lypolysis of the mitochondria. Its mean, that more energy resources could be used to increased of the sperm velocity. Sperm velocity of treatment of 4 mM dosage of caffeine was better than others treatment (P_0 : 47.20 μm/sec; P_2 : 55.99 μm/sec; P_6 (58,62 μm/sec) in top fraction and P_0 (48.6 μm/sec); P_2 (56,44 μm/sec), P_6 (58,29 μm/sec) in below fraction. Sperm velocity in treatment with supplementation of 6 mM dosage of caffeine in both of fraction (58.62 μm/sec in top and 58.29 μm/sec. in below fractions) could be decreased compared with 4 mM dosage of caffeine in top and below fractions (62.37 μm/sec and 64.24 μm/sec), it was better compared with control group (without caffeine). Lack of the balance of extender while higher of caffeine level and caffeine toxicities were influenced of the decrease of the sperm velocity. Caffeine have toxicity effect on nerve system and blood circulation (Richie, 1987 in Schunack, 1990). Sinha (1995) and El Gaafary et al. (1998) in Kubovicovan (2010) stated, that caffeine, when added to Tris-based diluent was beneficial for bull and goat sperm at doses of 10 or 20 mM, but this effect was dose-dependent and however higher doses caused a deleterious effect on spermatozoa.

By natural mating, spermatozoa was be in Tuba Fallopii in 15 minute. This sperm velocity could not take as a real standard of sperm velocity, beside from capability of their velocities, uterus contraction could be helped the motility of the spermatozoa in female reproductive tract (Gordon, 2005).

Conclusions

1. Supplementation of caffeine was significantly influence on sperm velocity of sperm separation of Garut Ram
2. Four (4) mM dosage of Caffeine was the optimum dosage to reach the highest motility and velocity of Garut Ram sperm separation both on top and below fractions.

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