

Effect of Glycerol Level in Two Different Extenders on Post Thawed Sperm Quality of Crossbreed Etawah Goat

Siti Darodjah Rasad, Soeparna, Nurcholidah, Rangga Setiawan and Rini Widyastuti
Animal Reproduction and AI Laboratory, Faculty of Animal Husbandry, Padjadjaran University,
Jatinangor, Indonesia

Abstract: Research about effect of glycerol level in two different extenders on post thawed sperm quality of crossbreed Etawah goat has conducted in breeding station and Animal Reproduction and AI Laboratory, Animal Husbandry Faculty Padjadjaran University. Aim of this research is to know the effect of glycerol level on sperm quality. This research used an experimental method with randomized block design. There were five type of treatment glycerol level (P1 = 5, P2 = 6, P3 = 7, P4 = 8 and P5 = 9%) and 5 Groups of goat with 2 replications. The 2 different extender were used as an extenders in frozen semen processing (egg yolk citrate and TRIS egg yolk). Research parameter of this research were motility, Intact Acrosome Cap (IAC) and Intact Plasma Membrane (IPM) post thawed sperm of crossbreed Etawah goat. As an conclusion; glycerol level affects on post thawing sperm quality of crossbreed Etawah sperm and the level of 6% glycerol is the optimum level to produced post thawed sperm quality, IAC and IPM which is qualified for AI program.

Key words: Glycerol level, egg yolk citrate, TRIS egg yolk, sperm quality, crossbreed Etawah goat, acrosome

INTRODUCTION

Freezing semen by cryopreservation as the best biotechnology of artificial insemination program. During cryopreservation technique, the semen has some advantages, although during the freezing and thawing processes induces certain detrimental effects in term of sperm structure, biochemical and functional damage (Nalley and Arifiantini, 2011). Some countries have already started artificial insemination in goats, although its commercial application is not extensive (Qureshi *et al.*, 2013). However, the main factor limiting more widespread use of frozen semen in caprine reproduction is the reduction of sperm viability during freezing processes (Batista *et al.*, 2009). Successful cryopreservation depends upon several factors including cooling rate, thawing rate, extender and dosis of cryoprotectants (Fernandez-Santos *et al.*, 2006).

The most commonly used cryopreservation diluents for goat semen have been either egg yolk or non-fat dried skim milk. However, goat sperm freezing diluents containing egg yolk or milk can be harmful to the sperm cells (Ajadi *et al.*, 2012). Egg yolk concentration is usually difficult to standardize in the most cryopreservation protocols for semen in various species (Fernandez-Santos *et al.*, 2006). Especially at high concentration addition of egg yolk to semen

extenders can be detrimental to spermatozoa (Julian *et al.*, 2006). During the freezing process will be form ice crystals that provide negatively affect to the spermatozoa. Cryoprotectants used for freezing of sperm cells provide protection from cold shock and the other damages during freezing. Optimum adding rates of cryoprotectant becomes peculiar to species and require determination of some parameters of cell membrane. Glycerol had extensively used as a cryoprotectant by Kulaksiz *et al.* (2010). Recent studies have demonstrated that glycerol remains to be the most effective cryoprotective compound for freezing goat semen and no enhancement was showed by the addition of other compounds by Kulaksiz *et al.* (2010). Therefore, glycerol is the most commonly used cryoprotectant for goat semen.

There are no more studies about different concentrations of glycerol for cryopreservation of goat semen by Kulaksiz *et al.* (2010). However, a lot of studies about glycerol concentrations had carried out about preservation of semen from different species (Pena *et al.*, 1998; Rota *et al.*, 1998; Baran and Ileri, 2000; Buhr *et al.*, 2001; Abbas and Andrabi, 2002; Rasul *et al.*, 2008; Awad, 2011; Hoffman *et al.*, 2011). Moreover, we did not find any study or other information about interaction of goat breed and also other species with glycerol concentrations on preservation of semen until now. There is no information on the cryopreservation of crossbreed of

Etawah goat semen and no study on the effect of different glycerol concentration on the preservation process of that breed has been reported. Therefore, the present study was designed to determine the effect of glycerol level in two different extenders on sperm quality of crossbreed Etawah goat.

MATERIALS AND METHODS

Research object: Semen as a research object was obtained from ten crossbreed Etawah goats (1.5-3 years old) and reared in breeding station of Animal Husbandry Faculty, Padjadjaran University.

Semen collection, dilution, freezing and thawing: Semen was collected from each goat twice a week on Monday and Thursday by means of artificial vagina. Immediately after collection, the ejaculates were placed in a water bath (37°C) and aliquots were taken for the assessment of semen quality. After individual examination, only ejaculates with at least 85% estimated progressive motility were used for freezing.

Two extenders were prepared as follows: Egg yolk citrate and TRIS Egg yolk Citrate added by glycerol 5, 6, 7, 8 and 9% each as a treatments.

The extended semen from groups (n = 10) was separately packaged in 0.25 mL straws and equilibrated at 4°C for 2 h. The straw was frozen in a styrofoam box at 4 cm above the Liquid Nitrogen (LN) surface for 15 min. The frozen semen was stored for 24 h in LN for further evaluations. The frozen semen straws from groups were thawed in a 37°C water bath for 30 sec and semen evaluation was carried out as follows.

Semen evaluation: The ejaculates were evaluated and accepted for evaluation if the following criteria which are: volume varying between 0.70-1.8 mL; sperm concentration of $1.95-4.75 \times 10^9$ sperm/mL; the motility percentage higher than 70 and <10% abnormal sperm in total.

Statistical analysis: The method in this research was Randomized Block Design (RBD) with 5 treatments and 10 Blocks of goat (each 1 goat every block) as replication so that they were obtained 50 experiment units.

Research parameter: Parameter of this research was motility, Intake Acrosome Cap (IAC) and Intake Plasma Membrane (IPM) post thawed sperm of crossbreed Etawah goat.

RESULTS

Result of this treatment on motility could be seen in Table 1. The level of glycerol could be effected to the sperm motility but there was the optimum level of glycerol. The optimum level of glycerol is 6%. It is the most optimal which was maintained life of goat sperm. Tambing *et al.* (2000) said during the freezing process, glycerol is able to penetrate the plasma membrane enters into spermatozoa cells and can be metabolized the energy and fructosa forming. Giving 6% glycerol into the citrate diluent is able to protect the spermatozoa from the effects of heat stress during the freezing process. Further, Tambing *et al.* (2000) explained that the effect of glycerol is to maintain balancing of intra and extracellular electrolytes so that the biochemical processes occurring within the spermatozoa cells could be persist and reduce spermatozoa cell damages during freezing process.

Result about Intake Acrosome Cap (IAC) in this research could be seen in Table 2. The best average of IAC value was in treatment 2 (with 6% glycerol). Addition of 6% glycerol in the yolk citrate diluent can provides the most optimal protection against the integrity of the acrosome of sperm after freezing because during the freezing process, the lipid peroxidation of spermatozoa was easy to damage the spermatozoa cells. The part of cell Spermatozoa is the most sensitive to endogenous peroxidative damage and the acrosome part was sensitive from exogenous peroxidative. The presence of glycerol that as a cryoprotective agent will maintain a balance of intra and extracellular physiologic concentrations and to protect acrosome caps of sperm (Tambing *et al.*, 2000).

Intact Plasma Membrane (IPM) sperm of crossbreed Etawah goats could be seen in Table 3. According to, Table 2 (Futino *et al.*, 2010) explained that role of glycerol in protecting the acrosome cap is also due to the protected of sperm plasma membrane. Table 3 showed that the addition of 6% glycerol could be the best result of the research compared to other treatments. The 6% glycerol level is the optimum dose which was giving the best value of intact plasma membrane. Glycerol will enter to the plasma membrane by balancing intra and extracellular concentrations. As a result, the water that had come out of the membrane by osmosis will reenter to the membrane and then the water Intra and extracellular content will balanced as same as before (Tambing *et al.*, 2000). Table 3, the lowest value of this glycerol is at the level of 9%, it is caused by toxic effects of glycerol (Rizal *et al.*, 2003). Toxicity effects occurred from glycerol by modification of structure from the plasma membrane and at high concentration of glycerol can be inhibited metabolism of the energy (McLaughlin *et al.*, 1992).

Table 1: Sperm motility in different level of glycerol

Groups	Glycerol level				
	G1 (5%)	G2 (6%)	G3 (7%)	G4 (8%)	G5 (9%)
1	38.42	46.74	42.27	36.76	31.66
2	35.46	45.83	41.21	37.97	32.46
3	32.05	42.71	40.00	40.84	35.90
4	41.43	46.97	41.43	36.54	30.63
5	37.80	45.05	36.61	38.76	31.06
Total	185.16	227.29	201.51	190.85	161.70
Average	37.03	45.46	40.30	38.17	32.34

Table 2: Average Intake Acrosome Cap (IAC) in different level of glycerol

Groups	Glycerol level				
	G1 (5%)	G2 (6%)	G3 (7%)	G4 (8%)	G5 (9%)
1	45.50	50.75	44.00	43.50	40.50
2	46.25	50.50	53.25	43.75	44.75
3	41.25	50.50	47.50	36.25	36.75
4	46.25	57.25	51.50	45.00	41.75
5	47.75	51.00	49.50	47.00	40.75
Total	227.00	260.00	245.75	215.50	204.50
Average	45.40	52.00	49.15	43.10	40.90

Table 3: Average Intact Plasma Membrane (IPM) in different level of glycerol

Groups	Glycerol level				
	G1 (5%)	G2 (6%)	G3 (7%)	G4 (8%)	G5 (9%)
1	54.00	63.00	55.50	46.25	43.50
2	50.50	65.50	62.25	46.25	41.25
3	46.25	59.50	52.50	48.50	40.25
4	58.25	66.25	58.25	51.50	45.75
5	57.50	62.00	58.25	51.50	49.50
Total	266.50	316.25	286.75	244.00	220.25
average	53.30	63.25	57.35	48.80	44.05

DISCUSSION

The highest average percentage of motility was obtained on the addition of glycerol level of 6% while the Lowest average percentage motility at the level glycerol of 9% (Table 1). This condition was happened that the additional of glycerol 6% optimal level in TRIS egg yolk citrate diluent and able to prevent the formation of ice crystals in the cells spermatozoa during the freezing process. Tambing *et al.* (2000) said in their research that the addition of glycerol 6% in tris diluent capable to providing the protection against sperm goats from adverse negative influenced for instant effect of modifying the protection that ice crystals formed during the freezing process, so that the damage of cell organelles spermatozoa can be avoided. Further, Tambing *et al.* (2000) represented that the protective effect is to maintain a balance intra and extra cellular electrolyte so that the biochemical processes that occur in sperm cells persist and reduce cell death spermatozoa excessive.

The low percentage of motility in addition of glycerol level of 5% because of that level was not optimal in a diluent while the addition of glycerol level of 7, 8 and 9% suspected have toxic effect from glycerol. Rizal *et al.* (2003) explained that the excessive concentration of glycerol that would cause toxic effects on spermatozoa, otherwise if less, glycerol will not provide the optimum effect. Level of glycerol could be effected of the osmotic pressure of the diluent turned toward hypertonic. Diluent that is hypertonic indicates that the molecules or particles outside cells more than inside the cell, resulting in the expenditure of water from inside to dilute the outside of cell where the cell will be shrinked (Mumu, 2009).

Based on Table 2, could be seen that the average percentage of IAC in treatment G2 post thawing with 6 % glycerol was higher (52.00%) than another treatments and the lowest average percentage at G5 with glycerol level 9% (40.90%). Level glycerol 6% giving the most optimal results against the integrity of sperm acrosome cap. This result was higher than the observation of Tambing *et al.* (2000) where the addition 6% glycerol provide average percentage of IAC 47.54 %.

The results of variance analysis showed that the level of glycerol in the diluent citrate egg yolk significant ($p < 0.05$) effect of IAC sperm of crossbreed Etawah goats post thawing. It mean that the glycerol level of 5 and 7% have not optimal working to maintain the integrity of the sperm acrosome cap (IAC) whereas the level of glycerol 8 and 9% showed a decrease in the IAC of crossbreed Etawah. The addition of glycerol 6% in diluent egg yolk citrate could be provide optimal protection against the integrity of the acrosome cap sperm after freezing because during the freezing process, spermatozoa was easy to perform of lipid peroxidation which were caused damage of sperm cells (Tambing *et al.*, 2000). Next Tambing *et al.* (2000) explained that part of cells spermatozoa is the most susceptible to damage and endogenous peroxidase exogenous is acrosome. Role of glycerol in protecting cap is also due to the acrosome membrane plasma that is protected (Futino *et al.*, 2010).

Result of Duncan's multiple range test showed that the level of glycerol 6% significantly ($p < 0.05$) better than other level (5, 7, 8 and 9%)(Table 3). Level of glycerol 9% is the lowest when compared with other treatments. This is in accordance with the statement of Rizal *et al.* (2003) stating that higher concentration of glycerol will cause toxic effect on spermatozoa whereas if it is less then the glycerol is not going to provide optimum effect. Sankai *et al.* (2001) stated that the percentage of IPM decrease occurred due to damage sperm plasma cause of

hardening membrane phospholipid layer due to low temperature. Addition of glycerol that can provide optimal protection for the survival of spermatozoa during the freezing process. Glycerol will get into the plasma membrane with the balancing of intra and extracellular concentrations. As a result, the water that had come out of the membrane by means extra osmosis will go back into the membrane and will further balance the intra and extracellular water content same as before glycerol enter (Tambing *et al.*, 2000).

CONCLUSION

Glycerol level affects on post thawing sperm quality, Intake Acrosome Cap (IAC) and Intake Plasma Membrane (IPM) of crossbreed Etawah goat sperm.

The level of 6% glycerol is the optimum level to produced post thawed sperm quality which is qualified for AI program.

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