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Effect of Sperm Concentration and Length of Storage at 5 ^{0}C on Motility of Goat Spermatozoa

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***Abstract*—**The objective of the present study was to determine the effect of different concentration of spermatozoa and length of storage in 5 ^{0}C on sperm motility. Semen was collected using artificial vagina from goat aged 2 to 2.5 years. Fresh goat semen with sperm motility ≥ 70% was used as material. Semen was divided into 4 treatments of concentration (40 x 10 ^{6} / ml, 50 x 10^6/ml, 60x10^6/ml, 70x10^6/ml) with length of storage 0,12,24,36 h. in 5 ^{0}C. There were interactions (P <0.05) between concentration and length of storage on individual motility of spermatozoa. Concentration of spermatozoa and length of storage affect the motility of individual (P <0.05). It was concluded that Sperm motility will decrease with increasing concentration and length of storage in 5 ^{0}C. Concentration of sperm 40x$10^{6/}$ ml and length of storage 0 h in 5^{0}C showed the highest motility of spermatozoa

***Keywords*—**Goat, Length of storage, Motility, Sperm Concentration

I. Introduction

ARTIFICIAL INSEMINATION (AI) is a reproductive technology to produce offspring in large numbers from a male superior.

The AI and semen cryopreservation.Technology have resolved the difficulty associated with transporting animals by means of transferring fresh or frozen semen over long distances Application of AI technique can use fresh semen, liquid semen, or frozen semen. Semen that has undergone processing with the principle of preservation can be stored in low temperature 5^{o}C and-196^{o}C. Frozen semen has been widely used commercially, but the lack of semen frozen was declined fertility[1]. This was due to sperm that had been frozen and thawing had been experiencing back some vitality capacitation so low and the cold shock which causes a decrease in motility and viability [2]. Previous results showed that although goat spermatozoa to maintain motility after freezing to thawing about 40-60%, but only about 10-30% who do not have biological damage [3]. The advantage of liquid semen was the process much easier, without the need for liquid nitrogen and the incidence of fertility decline in 5 ^{0}C can be minimized [2]. Besides the advantages of AI by using liquid semen was cheaper cost when compared to frozen semen. Liquid semen was stored at a temperature of 5^{0}C can be used 3-4 days [3].

The use of liquid semen can be used as a cheap solution, effective and efficient. But with the many advantages of liquid semen is still needed research on appropriate methods in liquid semen storage temperature 5^{0}C to increase the success of AI in the field.

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Sperm can not survive for long time unless added to various elements in the semen. Semen quality will decline rapidly during storage without extender [4]. Diluent provided nutrients for spermatozoa during the storage period, allowing spermatozoa to be able to keep moving progressive, non-toxic to spermatozoa, containing buffer and able to protect spermatozoa from cold shock.

Egg yolk tris amino methane has long been used in the semen diluent to provide protection to the extracellular spermatozoa during storage at low temperatures. Egg yolk diluent was added to provide protection of spermatozoa as a non-enzymatic anti-oxidants during the storage process [5]. This study aims to determine the effect of the concentration of spermatozoa and length of storage in 5 ^{0}C on sperm motility.

II. Materials And Methods

A. Semen Collection

Semen collected from goats aged from 2 to 2.5 years using an artificial vagina. Collecting semen was done once a week. Only samples with a minimum of 70% motile sperm and 80% morphologically normal spermatozoa were used in this research.

B. Semen Diluent

Semen diluent was Tris Amino Methane Egg Yolk.Compositition of Aminomethan Egg Yolk Tris diluent were : Tris 1.363 g , Citric Acid 0.762 g, lactose 1.5 g, 2.7 g Rafinose, fructose 0.5 g, penicillin 0.1 g and Streptomycin 0.1 g, egg yolk 20 ml,aquabidest 80 ml. Semen was divided into 4 treatments of concentr in 5 ^{0}C with length of storage 0,12,24,36 h.

C. Evaluation of motility and viability

Evaluation of individual motility of spermatozoa based on the percentage of motile spermatozoa. Evaluation of life and death sperm using eosin negrosin staining. Dead spermatozoa absorb dye while the live sperm do not absorb the color of eosin

D. Statistical Analysis

The study design was randomized block design with factorial pattern, 4 level of spermatozoa concentration as the primary factor and 4 levels of storage duration as the second factor. Each treatment was repeated 10 times. Data analysis using analysis of variance (Anova).

III. Results And Discussion

TABLE I
QUALITY OF FRESH SEMEN

Characteristic of Semen	Mean ($\bar{x} \pm SD$)
Volume (ml)	1.24±0.21
pH	6.82±0.06
Motility(%)	75.00±3.18
Viability (%)	80.10±2.70
Abnormality (%)	15.31±1.92
Concentration (10^6/ml)	2660.00±226.90

Volume of ejaculated semen were affected by age, condition of livestock, environment, food. Semen pH during the study was 6.82 ± 0.06 and the results can be considered normal.

That in general the normal pH was 6.8 till 7.0 and the sperms were very active. The percentage of viability and motility of fresh semen was used in this study were 80.10 ± 2.70% and 75.00 ± 3.18 % respectively. In any ejaculation there are a few abnormal spermatozoa, semen with a high proportion of abnormal spermatozoa will affect fertility. If more than 20% abnormal spermatozoa was indicated poor quality semen. Average concentration of spermatozoa were 2660.00 ± 226.90 million / ml. Concentration of spermatozoa need to know related to the dose and quality of semen produced after the handling of semen (freezing / cooling). As well as volume, sperm concentration varies not only between animals but also between species of livestock [7]. Based on observations of the characteristics of fresh semen was used feasible for further processing .

TABLE II
SPERM MOTILITY BASED ON DIFFERENT CONCENTRATION AND LENGTH OF STORAGE IN 5 ^{0}C

Sperm Concentration (10^6/ml)	Length of Storage (h)			
	0	12	24	36
40	70.50 ± 3.25^a	62.00 ± 3.31^a	52.32 ±3.45^a	43.55 ±3.22^a
50	70.25 ±4.11^a	58.25 ±3.20^b	48.25 ± 4.57^b	42.23 ±3.11^b
60	70.34 ±3.45^a	58.34 ±2.61^b	47.35 ±3.21^b	42.22± 3.05^b
70	70.24 ±4.55^a	55.22 ± 3.21^c	46.22 ± 3.43^c	40.65 ±2.54^c

Different superscripts in the same column indicate significant difference (P <0.05)

The results showed that the sperm concentration of 40 x 10^6 / ml and length of storage 0 h was the highest motility of spermatozoa. This is because of the higher concentration of spermatozoa in the consumption of nutrients can lead to rapid thinning out, while its availability was limited. Meanwhile, spermatozoa continue to metabolize and produce lactic acid that can kill sperm itself. [9]-[10]. For AI application, Indonesia National Standard (SNI) determines that the motility of spermatozoa should be ≥ 40%, so the individual motility of spermatozoa at various concentrations up to 36 hours was appropriate SNI standards. Another factor that decreases the motility of spermatozoa and fertility is the metabolism of free radicals that can damage sperm cells. Pospolipid free radicals will damage the cell membrane. Integrity of cell membranes are largely determine the process of sperm cell metabolism sperm motility depends on the energy supply in the form of adenosine tri phosphate (ATP) metabolism.

Metabolism will run well if the cell plasma membrane intact. The plasma membrane of cells play a role in regulating traffic in and out throughout the substrate and the electrolyte is needed in the process of cell metabolism [11]. The small number of cells in a unit volume, will cause the competitions the use of nutrients in the diluent can be minimized given that the metabolism of excess lactic acid can kill sperm. That during a certain time period, the metabolism of spermatozoa continue to run and produce waste products of metabolism of lactic acid. Lactic acid will experience a higher accumulation and result in damage to the spermatozoa was shown through the decrease in spermatozoa motility and durability. Semen quality decreases as the length of storage time.

There were interaction between concentration and length of storage on individual motility of spermatozoa (P <0.05). The higher sperm concentration and the longer storage in 5^0 C will decrease the motility. The influence of both factors could be due to the higher concentration of spermatozoa it will accelerate the expiration of the nutrients in a diluent in the consumption of the limited amount of diluent, so that more and more exhausted so that the spermatozoa are decreased energy and nutrient intake did not received[11]. During the shelf life of such a high number of spermatozoa concentration will produce the metabolism of lactic acid which is more that can kill sperm cells. In addition to the above factors, the storage in low temperature (5^0 C) is one of the factors affecting the decline of individual motility of spermatozoa. Due to the low-temperature storage can lead to cold shock on spermatozoa. Goat spermatozoa are sensitive to peroxidative damage due to the high content of unsaturated fatty acids in the phospholipids of the plasma membrane and the relative low antioxidant capacity of goat seminal plasma [12]. The formation of ROS generated by destruction of the plasma membrane caused a decrease in the ability of sperm motility and increase the damage that would affect morphology of sperm capacitation and acrosome reaction.[13]

In this research tris aminomethane egg yolk extender capable of maintaining individual motility of spermatozoa up to 36 hours. The egg yolk and its fractions are widely used because it provides protection for spermatozoa in semen when diluted or when the cooling and freezing of semen to a temperature of 0 ^{0} C[14]-[15]. In addition, egg yolks also act as an osmotic buffer that causes sperm cells are more tolerant of the diluent. Egg yolk had lipoprotein and lechitin which useful for maintained and protected the integrity of the cell of spermatozoa.

IV. Conclusion

Sperm motility will decrease with increasing concentration and length of storage in 5 ^{0}C. Sperm Concentration of 40x10$^{6/}$ml semen and length of storage 0 h in 5^{0}C showed the highest individual motility of spermatozoa. However, all levels of spermatozoa concentration and storage time to 36 hours in temperature 5 ^{0}C can still be used for AI

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