

Kidding rate of artificial insemination with Boer goat liquid semen during chilled preservation using coconut water-based diluent

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ABSTRACT: The quality of liquid semen with coconut water as diluent could be preserved up to 3 to 4 d during cold storage at 4 - 5°C. In this research, the successful insemination rate of coconut water-based diluent Boer goat liquid semen was observed to understand the application of coconut water as semen diluent. The materials used in this research were 5 to 7 months Viridis coconut water, liquid semen from 3 y old Boer buck, 30 does for acceptor, and Tris-aminomethane added with 10% egg yolk (EY) as a control diluent. The Boer semen was collected once a week by using artificial vagina, diluted and then stored before used for insemination. The insemination was done by using intra-cervical method at position 3, started with estrous synchronization by using 2 mL PGF2 α . The research was conducted as experimental method under randomized group design with two treatments, that were Tris-aminomethane + 10% EY (P0) and coconut water + 10% EY (P1). All of the treatments were repeated for 15 times and the collected data were analyzed with Pearson's chi square with Genstat 18 program. The observed variables include non-return rate and kidding rate. The results showed that the first non-return rate of P1 were 93.33% and in P0 were 73.33%, while the second non-return rate of P1 were 93.33% and in P0 were 80%. Furthermore, the kidding rate of P1 were 40% and P0 were 66.66%. The Pearson's chi square analysis showed that there was no effect of coconut water as semen diluent to the kidding rate. The research concludes that the usage of coconut water as semen diluent did not affect the successful insemination rate of Boer goat.

Keywords: Kidding Rate; Cocos nucifera; Boer

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INTRODUCTION

The reared Boer goat breed in Indonesia is originated from Africa, as the breed has several superiorities as meat producer, such as its adaptability to be reared in tropical area aside of its meat production. The development of Boer goat breeding in Indonesia is also supported with the vast grazing area, feed availability, market potential and suitable climate in the country. The distribution of Boer breed goat across the country should be done by using artificial insemination technology of chilled Boer semen to tackle obstacles such as geographical hindrances due to the archipelagic condition of Indonesia.

An approach to preserve semen quality during cold preservation has become widely research interest, and of the promising approach is by using semen diluent which had antioxidant properties. Coconut (*Cocos nucifera.L*) water has shown its potential to be used as semen diluent as it contained beneficial biochemical compounds for semen diluent during 5°C preservation, and added with abundant availability in Indonesia as well. Cardoso *et al.* (2005) and Yong *et al.* (2009) showed that the usage of coconut water as semen diluent would play as buffering agent for the semen, and is cheap, practical and effective for application. Blume and Marques (1994) added that coconut water contained salt, protein, sugar, vitamin, fat and various electrolytes that provide nutrients for the semen.

Coconut water also contained micronutrients such as organic ions and vitamins that play as antioxidant and prevent reactive oxygen species due to the cell hypermetabolism (Evans and Halliwell, 2001). Other compounds in coconut water are sorbitol, lipid, amino acids, nitrogen compounds, organic acids and various enzymes (Chugh *et al.*, 2009). The antioxidant compounds in coconut water, such as vitamin C and flavonoid, would protect spermatozoa from lipid peroxidation during cold storage by releasing one

hydrogen atom to bind free radicals which cause membrane disruption through certain chain reaction. The addition of coconut water is then expected to maintain semen motility and viability which will ensure the successful insemination. Research by Daramola *et al.* (2016) on West African Dwarf goats showed that the addition of coconut water and 10% egg yolk could protect spermatozoa during cold storage.

The research on the utilization of coconut water as semen diluent has been widely conducted on various animals, however the utilization on Boer goats has not been done, especially up until the insemination. This research aimed to observe the kidding rate of cold stored Boer semen insemination by using coconut water as semen diluent. The result of this research is then expected to elucidate novel protocols and methods to deliver cold stored Boer goat semen to be distributed on the rural area.

MATERIALS AND METHODS

Materials

The research was conducted for 10 months from semen preparation, insemination and kidding delivery. The biochemical properties of coconut water were done on the Laboratory of Biochemistry, Faculty of Mathematics and Science and Laboratory of Food Quality and Safety, Faculty of Agricultural Technology, University of Brawijaya. The semen was prepared and analyzed on the Laboratory of Animal Reproduction, Faculty of Animal Science, University of Brawijaya. The pregnancy and kidding test were conducted on the CV. Agriranch goat farm, Malang, Indonesia. The materials used in this study were 6-months unripe *Viridis* coconut water. The control group in this research was made by using Tris-aminomethane and 10% egg yolk. The 3 y old Boer semen was collected once a week by using artificial vagina. Other materials for analysis were bicnat buffer, 3% NaCl, eosin-negrosin, penicillin, streptomycin, 0.22 mm Whatman filter paper, PGF2 α (Lutaprost, Peru), and mini

straw. As much as 30 does were inseminated in this research, which were 2.5 to 4.5 y old Ettawah crossbreed goats weighed at 30 to 40 kg.

Methods

The research was conducted with two treatments and 15 replications. The treatments in this study were Boer goat semen added with Tris-aminomethane and 10% egg yolk as control group (P0) and Boer goat semen added with coconut water and 10% egg yolk. The Boer goat semen was firstly cold stored at 4-5 °C for 24 h, and then analyzed for its individual motility. The semen with at least 40% motility were then used in this research.

The estrous synchronization was done 72 h before inseminated by using 2 mL of PGF2α for each doe. The estrous synchronization was done through subcutaneous injection after observed by USG (Draminsky, Poland) to determine the doe’s pregnancy status. The insemination was done by using insemination gun and semen deposition was by following intra-cervical method position 3. The observed variables were non-return rate (NRR) and kidding rate (KR). The NRR percentage was measured by determining the proportion of

cows that are not subsequently re-bred within a specified period of time after an insemination, while the KR percentage was measured by determining the proportion of pregnant does after insemination.

Data analysis

The research was conducted in randomized group design and the collected data were analyzed with Pearsons’ chi square (Steel and Torrie, 1995) by using Genstat program to determine the non-return rate and kidding rate.

RESULT AND DISCUSSION

The biochemical analysis showed that coconut (*Cocos nucifera* L) water contained various nutrients. The glucose on the coconut water could provide energy for mitochondria through Adenosine Tri Phosphate (ATP) production which improve spermatozoa motility and maintain both intracellular and extracellular osmolarity that protect spermatozoa during cold storage. According to St. John (2002), the mitochondrial ATP would initiate fibril contraction that cause spermatozoa motility. Roberts *et al.* (2013) added that the motoric activity of spermatozoa is through its dynein axonema tail.

Table 1. The biochemical properties of several coconut water varieties

PARAMETER	COCONUT WATER			
	VIRIDIS		RUBESCENS	
	Unripe	Ripe	Unripe	Ripe
Glucose (%)	0.49 ± 0.02	1.00 ± 0.00	0.49 ± 0.01	0.07 ± 0.00
Protein (%)	0.22 ± 0.00	0.13 ± 0.00	0.20 ± 0.00	0.13 ± 0.00
Fat (%)	0.044	0.10	0.042	0.14
Vitamin C (mg/100 ml)	0.00	5.15	0.00	2.60
Fe (mg/L)	0.05 ± 0.00	0.12 ± 0.00	0.07 ± 0.00	0.07 ± 0.00
Mg (mg/L)	290.770 ± 0.00	262.50 ± 0.05	212.64 ± 0.05	258.20 ± 0.05
Na (%)	0.12 ± 0.00	0.80 ± 0.00	0.10 ± 0.00	0.63 ± 0.00
Zn (mg/L)	0.66 ± 0.00	0.30 ± 0.00	1.13 ± 0.00	0.15 ± 0.00
K (ppm)	759.03	686.58	1116.16	578.08
Ca (ppm)	71.10	24.48	90.61	37.81
Antioxidant IC ₅₀ (mg/ml)	2539.00	494.00	1321.50	2487.00
Viscosity (cP)	2	1.00	2	2

Glucose would also initiate spermatozoa hyperactivity (Portela *et al.*, 2015), indicated with progressive

movement. According to Susilawati (2011), the spermatozoa hyperactivity is indicated with the rapid changes of moving pattern,

formed whiplash on its flagellum, increased curve linear velocity and decreased linearity.

Aside from glucose, the calcium (Ca) and bicarbonate (HCO₃) would also contribute to the spermatozoa hyperactivation. Ho and Suarez (2001) reported the physiological hyperactivation due to the Ca and HCO₃ on the semen diluent, while Chen (2000) added that the condition is through adenylyl cyclic modulation in the flagella. The hyperactivation of spermatozoa thus fasten its motility into ovum and caused fertilization and pregnancy. The fertility is then could be measured by determining non-return rate and kidding rate after insemination as presented in Figure 1 and 2.

Non-Return Rate (NRR)

The statistical analysis showed that the NRR of Boer goat was not significantly different both on P1 and P0, with each reached 73.33% and 80% on first NRR and 93.33% and 93% during second NRR, respectively. The results showed that the usage of coconut water and Tris-aminomethane as semen diluent was

relatively similar. This indicates that the usage of coconut water as semen diluent for cold stored Boer semen could be applied. The condition is due to the availability of glucose and calcium on the coconut water which provide energy and initiate hyperactivation of spermatozoa and maintain the semen quality which resulted good NRR.

The usage of coconut water as semen diluent in this research is better than milk (*laichipos*) on Norwegian goat which showed 87% NRR as reported by Paulenz *et al.* (2005). There several factors which affect the obtained NRR in this research, which are individual spermatozoa motility that reached 65% with 50 million spermatozoa/straw concentration, and total spermatozoa motility which was more than 80 million/mL before insemination. Both factors were then contributing to the fertilization on fallopian tube isthmus and its ability to penetrate pellucida zone. According to Susilawati (2011 and 2013), the spermatozoa motility and concentration are essential requirements for fertilization through artificial insemination.

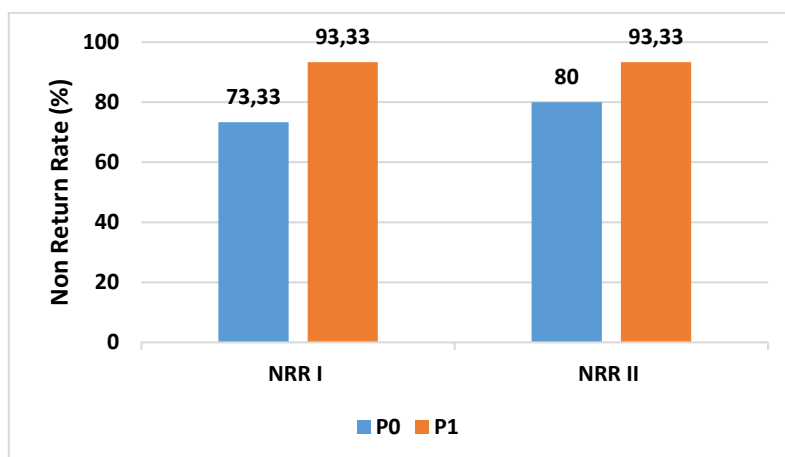


Figure 1. Non-Return Rate

Moreover, energy supply from glucose in the coconut water provide mitochondrial ATP to stimulate spermatozoa movement. Wiliam and Ford (2001) also described that spermatozo of mammals require metabolic energy for its motility, while Rodriques-Gill (2006) showed

that seminally plasm did not contain glucose for energy supply, except on pig semen.

Kidding Rate

The Pearson's square statistical analysis showed that there was no significant difference on kidding rate of P0 and P1 insemination. The research showed

that P1 had 40% kidding rate, while P0 had 66.66% kidding rate of 15 inseminated does. The kidding rate in this research was lower compared to the finding by Paulenz *et al.* (2005) on Norwegian goat insemination with Laichipos diluent, which had 78% kidding rate. This indicates that the usage of coconut water as semen diluent of cold stored Boer semen did not affect the kidding rate. However, the statistical analysis

showed that the usage of Tris-aminomethane as semen diluent had higher kidding rate. The condition is caused by the higher spermatozoa endurance after diluted with Tris-aminomethane, thus provide better spermatozoa motility. Furthermore, two NRR periods measurement showed that there was asymmetric NRR and kidding rate of both Tris-aminomethane and coconut water as semen diluent.

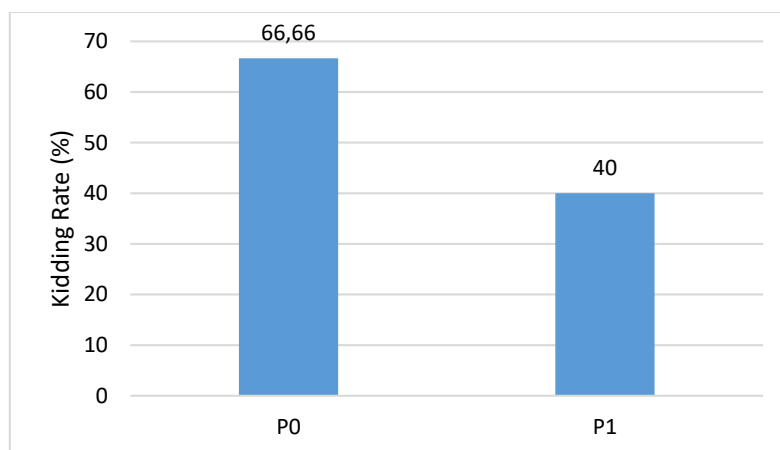


Figure 2. Kidding Rate

The NRR of coconut water (93.33% and 93.33%) and Tris-aminomethane (73.33% and 80%) as semen diluent were not linear to the kidding rate of both diluents, which were 40% and 66.66%, respectively. The asymmetric results were due to the undetected failed fertilization of does that were inseminated with coconut water diluted Boer semen. The failed fertilization is affected by several factors, such as the decreased spermatozoa physiology due to suspension on coconut water, temperature, or pH changes. In accordance, Flesch *et al.* (2000) also added that the decreased cell membrane function correlate to the fertilization.

CONCLUSIONS

The usage of coconut water as semen diluent did not affect the successful insemination rate on Boer goat.

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