RESEARCH PAPER

Sperm Cell Livability and Motility from Saanen Buck Extended with Coconut (Cocos nucifera L.) Milk and Skim Milk at Room Temperature

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Abstract

In the Philippines, there has been an increased interest in discovering the novel semen extenders for small ruminants and the duration of sustaining the motility of spermatozoa. The study aimed to determine and compare the suitable semen extender for Saanen buck spermatozoa at room temperature. The treatments were coconut milk-based extenders; skim milk-based extenders and non-addition of extender to semen. Semen was collected from a healthy Saanen bucks with no andrological defects using the artificial vagina (AV) method to determine the motility and livability for 1, 2, 3, 4, and 5 hours at room temperature. Results revealed that percentage motility of coconut milk and skim milk-based extenders are comparable after an hour. Percentage motility of coconut milk-based extender is significantly higher than non-addition of extenders from 1 to 5 hours observation period. The sperm motility and livability decreased drastically after 3 hours in all treatments. Therefore, the coconut milk-based extender appears to be a better option compared to the skim milk-based extender, and non-addition of extender for semen of Saanen bucks. These extenders must be used within 2 to 3 hours because this is the period found to possess higher motility percentage. Whether the differences found between the extenders will be reflected in the fertility results after artificial insemination (AI) is yet unknown and needs to be further studied.

Keywords: *Coconut Milk, Motility, Saanen Buck, Semen extender, and Spermatozoa*

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Introduction

Artificial insemination (A.I.) in livestock especially in goat is one of the labor-saving breeding method being used to improve the Philippine animal production and productivity. It was also introduced by Philips and Lardy 1939; Salisbury et al 1949 and Sule et al 2007 that there is an increasing need for the use of A.I. in goats (Oyeyemi et al 2001 and Sule et al 2007); the needs of novel extender for semen is necessary to make A.I. economically beneficial. However, the success of A.I. is based on the ability to expertly collect and maintain the viability of spermatozoa from quality of male goats and use in inseminating to the female goats.

According to Langford et al 1979; Hacket and Wolynetz 1981, goats spermatozoa can be extended through the use of different methods it can be frozen thawed, chilled, or fresh (i.e. non-refrigerated), but when fresh semen is used, it offers better fertility and conception rate (Langford et al 1979; Hacket and Wolynetz 1981). In addition, Blash et al 2000, stated that the process of freezing and thawing of goat semen reduces the percentage of live sperm cells and acrosomal integrity. To meet the need for A.I., many extenders have been used and these are egg yolk-phosphate (Phillips and Lardy 1940), skim milk (Almquist and Wickersham 1962), and coconut milk.

Coconut (*Cocos nucifera*) milk was first used as part of an extender for semen by Norman in 1962 for keeping the livability of fresh semen. Coconut milk was also reported by Melo and Nunes 1991 to give appreciable sperm cell's motility and fertility with an

acceptable conception rate (CR) in cattle when semen used fresh (Grove and Lewis 1965).

Semen quality and its relationship to fertility are said to be major concerns in animal production, hence an accurate measurement of semen fertilizing potential is of great importance (Grasa et al 2004 and Sule et al 2007). The utilization of novel extenders, actual proportion and suitable length of time for extension of buck semen has not been documented in Bohol, Philippines. Hence, this study was to assess the suitable semen extender for Saanen buck spermatozoa and to determine the sustaining time of the motility in studied extenders at room temperature (37 degrees Celsius).

Methodology

Research Design and Treatment

The semen was collected and divided into three equal parts then assigned to the different treatments following the Completely Randomized Design. Five replications were generated per treatment. The replications were the five motility readings taken from each test tube with or without extended semen. The following treatments were used: Treatment 1 - Buck's semen with coconut milk-based extender, Treatment 2 - Buck's semen with skim milk-based extender, Treatment 3 - Buck's semen with non-addition of extender.

The study was conducted to evaluate the semen volume, color, pH, sperm cell motility, and longevity in different treatments (Coconut milk-based extender, Skim milk-based extender, Non-addition of extender).

Research Environment

The study was conducted at the University Small Ruminants Project and in the Reproduction Laboratory of the College of Agriculture and Natural Resources, Bohol Island State University-Bilar Campus, Bohol Island, Philippines.

Animals and management

The two fertile and clinically healthy matured Saanen bucks (3-year-old) with an average weight of 60 kilograms was used in this study and were preliminarily examined during the acclimatization of the bucks. The buck was housed in a well-illuminated pen that has a wooden floor raised above the ground level. The necessary arrangement for feeding and free access to fresh water (*ad libitum*). The bucks were fed grass twice daily and concentrate supplements in the evenings with a diet consisting of rations. The general management program including disease prevention in the project was followed. The bucks have already been trained to mount on the artificial dummy for easy semen collection.

Extender Preparation

Coconut Milk

Mature ripe coconut was split open and the white coconut flesh was removed from the shell using a clean sterilized knife. The coconut flesh was cut into bits and blended, without the addition of water, in a clean electric blending machine. The blending was done twice. The finely blended flesh was wrapped in a clean sterilized white handkerchief. This was squeezed manually to extrude the juice. The coconut juice thereby collected was dispensed into clean sterilized centrifuge glass tubes, tightly capped, and centrifuged at 500 rpm for 20 minutes (Sule et al 2007). Coconut milk was carefully sucked up the oily layer of the spun coconut juice using a new needle syringe and then centrifuged for the second time to remove shaft and oiliness of the coconut milk. Following this, the required volumes and pH of coconut milk were measured and recorded after that the collected coconut milk were dispensed in a 5 clean and sterilized tubes. Then, the tubes were tightly capped and kept at 37^{o}C through a water bath until used for semen extension.

Skim Milk

The Skim Milk-based extender was prepared from non-fatty milk powder and distilled water, heated at 95^{0}C for 10 minutes, and after cooling to room temperature. Following this, requisite volumes of skim milk were dispensed into 5 clean and sterilized semen collection tube and labeled accordingly. After that, tubes were tightly capped and kept at 37^{o}C through a water bath until used for semen extension. The pH and other parameters of each extender were measured and recorded.

Semen Collection

The collection of Sannen buck semen was made through the artificial vagina (AV) method with optimum pressure and temperature of 37 degrees Celsius in a graduated collection tube. This activity was done at 7:00 A.M. The buck was allowed to mount on the artificial wood dummy goat at least 1-2 false mounts. After mounting was complete (with seeking movement of the penis), the buck made vigorous upward and forward thrust to the artificial vagina assembly which signifies the occurrence of ejaculation. The tube containing freshly collected semen was immediately transferred to the ice box with

an appropriate temperature level. The semen was not exposed to any unfavorable conditions during or after collection.

Evaluation of Semen Motility

Mayayashi, (1968) constructed the criteria for determining motility and adopted them by Magalso, 2008 and Chua et al 2021. With these, characterizing the treatments would be easier. The motility readings in this study were referred to this criteria. Excellent motility- 80% or more spermatozoa are in vigorous motion; Swirls and "Eddies" caused by movements of the sperm are extremely rapid and constantly changing. Very good motility- approximately 70-80% of the spermatozoa are in vigorous motion. Waves and "Eddies" drop rapidly but not as in excellent motility. Good motility- about 50-70% of the spermatozoa are motion as vigorous but the waves and "Eddies" form more slowly across the field. Fair motility- more than 30% of the sperm are in motion. The movement is fairly vigorous. Poor motility- less than 20% of the sperm are already dead. The movement is very weak and not progressive or impotent. In the semen motility assessment, a requisite amount of semen was taken from the beaker and placed in a glass slide covered with a cover slip. This was taken at the mounted stage in the stage of an electric microscope and evaluated for motility with the subjective motility reading based on Mayayashi's criteria. Five replications per treatment were used to check the hourly motility of three treatments. If 0% was observed in a certain treatment, the evaluation was then marked and recorded.

Pre-extension evaluation of semen

This protocol used in this study was adopted by the work of Sule et al 2007, after semen collection, an amount of semen from the buck's prepuce was put on a clean warm glass slide and assess the mass activity of the semen and the values recorded. Semen concentration and motility was quickly done through microscopic evaluation. In each trial, before semen extension, volume, motility, and concentration of semen were quickly assessed and the values were recorded as pre-extension semen parameters.

Semen extension

A similar study by Ajala et al 1997 was adopted in this method, the semen was extended by dispensing 0.5 ml semen into 0.5 ml coconut milk-based extender; 0.5 ml skim milk-based extender, -and a non-addition extender previously kept at 37^{o}C. Semen motility was assessed and recorded. Each tube of extended semen was thereafter, stored in a room temperature and kept away from sunlight for 1, 2, 3, 4, 5 hours sperm cell motility assessment.

Evaluation of Post-extension of semen motility

In this method, similar study done by Ajala et al 1997 for a pawpaw juice extender. The post-extension sperm cell progressive motility was assessed and recorded an hourly motility score in percentage (%) using Mayayashis creteria. The motility assessment was done rapidly and meticulously to ensure little time lag in assessing other extended semen. The concentration of motile extended sperm cells of the semen was estimated for the most suitable extender using the average pre-extension semen volume and concentration with average post-extension motility at 5 hours storage time, and the values recorded.

Statistical Analysis

Data were entered in Microsoft Office Excel 2016 program and sorted, and descriptive statistical was done to get the Mean $\pm$ SEM value. One-way analysis of variance (ANOVA) and comparison of treatment means was done by the least significant difference (LSD) test using the Statistical Tool for Agricultural Research (STAR) version 2.0.1.

Results and Discussion

The attributes of white and black buck semen are as shown in **Table 1a** white buck and **Table 1b** black buck. The data was collected during the acclimatization period. The results revealed (**Table 1a and 1b**) that the motility score of sperm cells of the T_1 was significantly higher *($p<0.02$)* than T_2. Semen color is the indication sperm cell concentration were white buck (T_1) observed milky-white in color **(Table 1a)** while it was consistently watery for the (T_1) black buck **(Table 1b)** this means black buck has a poor sperm cell concentration. This was supported by the mean concentration of sperm cells of the white buck which was significantly higher *($p<0.02$)* than the black buck's **(Tables 1a and 1b).**

Table 1a: Semen Attributes of White Buck (T_1) during Acclimatization.

Semen	Number of Trials					
	1	2	3	4	5	Mean $\pm$ SEM
Volume (ml)	0.5	0.4	0.5	0.4	0.5	**0.46**

						± 0.02
Mass Activity (0-4)	4	4	4	4	4	4
Concentration of sperm cells (billion per ml)	0.22	0.17	0.17	0.25	0.07	0.26 ± 0.02
Progressive Motility (%)	94	94	95	93	92	93.6 ± 0.51
Live (%)	97.2	97.4	98.2	97.12	97.3	97.44 ± 0.19
Color	Milky white	Milky white	Milky white	Milky white	Milky white	Milky White

Table 1b: Semen Attributes of Black Buck (T_2) during Acclimatization.

Semen	Number of Trials					
	1	2	3	4	5	Mean ± SEM
Volume (ml)	0.3	0.5	0.3	0.31	0.6	0.45 ± 0.06
Mass Activity (0-4)	1	2	1	1	2	1.4 ± 0.22
Concentration of sperm cells (billion per ml)	2.77	1.22	1.74	1.58	1.52	1.77 ± 0.27
Progressive Motility (%)	50	45	45	43	42	45 ± 1.23
Live (%)	84	96.1	85	90	93.1	89.64 ± 2.07
Color	Watery	Watery	Watery	Watery	Watery	Watery

Values of Coconut milk-Based Extender and Skim milk-Based Extender

The requisite amount of coconut milk-based extender obtained in the five (5) trials as well the requisite amount of coconut milk-based extender at 1st, 2nd , 3rd, 4th and 5th hour reading and the mean pH is shown in **Table 2a** .The pH of the coconut milk- based extender ranked from 6.4 to 8.6 with an average 7.5 ± 0.35 Mean ± SEM. However, the pH of skim milk-based extender ranked from 6.1 to 7.8 with an average of 6.86 ± 0.35 Mean ± SEM. The requisite amount of skim milk-based extender at 1st, 2nd, 3rd, 4th and 5th hour reading and mean pH are shown in **Table 2b.**

Table 2a. The amount of coconut milk-based extender, and mean pH

Hourly Period	Coconut Milk-Based Extender		Total Volume	Semen Volume	Dilution ratio	Mean pH
	ml	%	ml	ml		
1hr	0.5	100%	0.5	0.10	1:6	6.4
2hrs	0.5	100%	0.5	0.10	1:6	6.9
3hrs	0.5	100%	0.5	0.10	1:6	7.6
4hrs	0.5	100%	0.5	0.10	1:6	8
5hrs	0.5	100%	0.5	0.10	1:6	8.6
Mean ± SEM						*7.5 ± 0.35*

The requisite volume of 0.5 ml based extenders contained the volume of 0.10 ml viable or motile semen, which probably sufficient for artificial insemination in animals; according to Sule et al 2007; Pagot, 1993; and Zemjanis, 1977 the requisite 0.5 ml extended semen and the requisite amount of viable or motile semen contained could be a good insemination ratio for does through the intra-cervical route. However, Roberts, 1971 and Sule et al 2007 and workers in this field was recommended the higher volume of extender and volume of spermatozoa to obtain good fertility in animals.

Table 2b. The amount of Skim milk-based extender, and mean pH

Hourly Period	Skim Milk-Based Extender		Total Volume	Semen Volume	Dilution ratio	Mean pH
	ml	%	ml	ml	ml	
1hr	0.5	100%	0.5	0.10	1:6	6.1
2hrs	0.5	100%	0.5	0.10	1:6	6.3
3hrs	0.5	100%	0.5	0.10	1:6	6.5
4hrs	0.5	100%	0.5	0.10	1:6	7.6
5hrs	0.5	100%	0.5	0.10	1:6	7.8
Mean ± SEM						*6.86 ± 0.35*

The Motility Scores (%) of Sperm Cells at Different Extenders (hourly period reading) at Room Temperature 37 ^{0}C

The results show (**Table 3**) that the treatment that contained 0.5 ml coconut milk-based extender had the highest motility rate of 79.80% described it as very good motility an hour after the buck's extended semen stored at 37^{o}C compared with the skim-milk-based extender with 69.60% while the semen with non-addition of extender has the least motility of 20%. The better performance of the coconut milk-based extender of buck semen may be due to the high amount of unique healthy chemicals and the active ingredient present in the coconut milk which is the antibiotic.

It was also observed that among all treatments, coconut milk-based extender has a highly significant difference from the 1st, 2^{nd,} and 3rd-hour interval until the 4th hour with a similar trend whereby there was a fast reduction of sperm cells motility of buck semen with non-addition of extender, then this was followed by the skim milk-based extender. The essence of an extender is not only to add volume to the semen but also to provide nutrients to the sperm cells to replenish what it used up to maintain its life outside the reproductive tract (Brinsko et al 2011).

Five hours after extending the buck's semen, coconut milk-based extender, skim milk-based extender, and non-addition of extender at 37 degrees Celsius showed a 20% to 7.60% motility rate indicating that all sperm cells were already dead and the movement is very weak and impotent.

Table 3. The Motility Scores (%) of Sperm Cells at Different Extenders at Room Temperature (Hourly period)

Treatments	Hourly Period Reading				
	1st Hour	2nd Hour	3rd Hour	4th Hour	5th Hour
T_1 - Coconut Milk-Based Extender	79.80	69.60	60	52.71	49.80
T_2 - Skim Milk-Based Extender	69.60	49.80	37	35	27.60
T_3 – Non-Addition of Extender	20	20	20	16.40	7.60
P-value	***0.0001****	***0.0001****	***0.0001****	***0.0001****	***0.0001****
CV (%)	*0.72*	*0.88*	*9.93*	*11.67*	*15.25*

Means within the column with dissimilar letter superscripts are significantly different (p < 0.05).

Statistical analysis of the result showed that there is a highly significant difference of *(p<0.0001)* among the treatments. Thus, the results of this study revealed that coconut milk-based extenders and skim milk-based extenders are comparable since their difference in the percentage of motility is not far in number. However, coconut milk – based extender is highly significant *(p<0.0001)* from the treatment with non-addition of the extender. One factor; that might be the reason for the observed decreasing percentage of sperm cells' motility was the non-addition of antibiotics in the treatment. This probably allowed the rapid reduction of sugar by the competing microbes. This probably the reason affect the treatments of semen with a non-addition extender in which after 1st hour of semen extension the motility percentage score dropped from 20% to 7.60% value for non-addition of extender in all periods. In addition of antibiotics, the study by Sule et al (2007), reported that coconut milk-based extender and skim milk-based extender has antibiotic medium this means the inclusion of antibiotics hence recommended to improved and extended the usefulness of the semen stored at room temperature (37 degrees Celsius).

Conclusion and recommendation

The experiment on the coconut milk-based extender; skim milk-based extender and non-addition of extender for Saanen buck goat spermatozoa at room temperature (37 ^{0}C) revealed that coconut milk- based extender and skim milk-based extender has a comparable result in terms of percentage motility that ranges from 80% - 70% in the 1st hour. However, the coconut milk-based extender is significantly *(p<0.0001)* higher than non-addition of extender from 1 to 3 hours. Results also revealed that motility and livability decreased drastically after 3 hours in all treatments. Therefore, the coconut milk-based extender appears to be a better option compared to the skim milk-based extender and the non-addition of extender for semen of Saanen buck after 2 to 3 hours with highly significant *(p<0.0001)* in motility percentage. Whether the differences found between the extenders will be reflected in the fertility results after artificial insemination (AI) is yet unknown and needs to be further studied.

Ethical approval

The experiments performed in this study were according to the rules of the Animal Welfare Act of 1998 (RA 8485) in the Philippines. The researchers secured Institutional Animal Care Use Certificate (IACUC) before the conduct of the study.

Competing interests

The authors have declared that no competing interests exist.

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