



The Influence of a Tropical Climate with a Long Dry Season on Goat Semen Quality and Seminal Fluid Change in the Kanchanaburi Province, Thailand

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ABSTRACT

At present, there is no information about the difference between the three seasons with a long dry period, such as Kanchanaburi province, Thailand, on goat semen quality. This study observed the climate and daytime length effects of a long dry period in three seasons in a tropical climate country on goat semen quality and seminal fluid change. The semen was collected from eight male goats once a month for one year. The libido score was assessed during semen collection. Semen volume, semen sediment volume, percentage of seminal fluid volume, seminal fluid protein, mass movement, motility, concentration, and spermatozoa membrane integrity were evaluated. Libido scores were not different between the seasons. Semen volume, seminal fluid volume, and seminal fluid protein concentration in the rainy season were significantly higher ($p < 0.05$) than in the summer and winter. Semen sediment volume was relatively stable in all seasons and months. Meanwhile, the concentration was significantly higher ($p < 0.05$) in the summer and winter than in the rainy season. In summer and winter, spermatozoa motility and normal spermatozoa membrane were significantly higher ($p < 0.05$) than in the rainy season. In conclusion, temperature, THI, and daytime changes in tropical climate countries with long dry seasons were unrelated to semen quality, seminal fluid change, and sexual behavior. But the increase in humidity in the rainy season after long dry seasons has dropped semen quality, resulting from increased volume and protein levels in seminal fluid.

Keywords: goat, long dry season, semen, seminal fluid, tropical climate

INTRODUCTION

A seasonal variation in semen quality has an impact on frozen sperm production. The study of seasonal factors affecting goat semen quality allows us to stabilize goat quality throughout the year. Seasonal variations in goat sperm quality have been reported in tropical climate countries (Aguiar *et al.*, 2013; Dias *et al.*, 2017; Isnaini *et al.*, 2020). Brazil's Northeast was a tropical climate country that had goat semen quality improvement in the rainy season (Aguiar *et al.*, 2013; Salles *et al.*, 2020). According to goat semen in Indonesia, semen quality has increased in the rainy season (Isnaini *et al.*, 2020). However, the two regions of study in Brazil and Indonesia have two main seasons (dry and rainy seasons), and the periods between the two seasons are not different (da Silva, 2004; Isnaini *et al.*, 2020). Thailand's western region has a tropical savanna climate with three seasons (summer, rainy, and winter) (Thai

Meteorological Department, 2015). This region has a continuous dry season during the winter and summer (Thai Meteorological Department, 2015). Seasonal differences between Thailand and the previous two countries may have affected the goat semen quality in different ways. Goat seminal fluid protein varied in each season and showed different effects on semen quality (Arrebola & Abecia, 2017; Teixeira *et al.*, 2018). The effects of Thailand's long dry seasons on goat sperm quality, seminal fluid volume, and seminal fluid protein have never been studied.

The climate and the photoperiod in each season have influenced the male goat semen (Dias *et al.*, 2017; Isnaini *et al.*, 2020). The climate factors affecting reproductive function are temperature, humidity, and the photoperiod. The temperature and humidity index (THI) was used to evaluate heat stress that affected goat semen quality (Isnaini *et al.*, 2020; Ranjan *et al.*, 2020). High THI could reduce goat semen quality. The climate

of the 3 seasons in Thailand has different temperatures, humidity, and THI. Moreover, the photoperiod (or day-time) is a key factor in changing the goat semen quality. Goats had semen quality improvement in a short-day season (Gallego-Calvo *et al.*, 2015). Kanchanaburi province in Thailand, located above the equator line, has long dry seasons (summer and winter) and little difference between day and night duration throughout the year. At present, there is no information about the difference between the three seasons with a long dry period on goat semen quality. This study observed the climate and daytime length effects of a long dry period in three seasons in a tropical climate country on goat semen quality and seminal fluid change.

MATERIALS AND METHODS

Animals and Place of Study

This protocol was approved by the Animal Usage and Ethics Committee of Veterinary Science Faculty, Mahidol University (ID no. MUVS-KA-2020-01-01). Throughout the experiment, eight male goats of mixed breed (Native and Boer) (*Capra hircus*) aged 3 to 4 years and weighing 30 to 45 kg were kept in the Research house at Livestock and Wildlife Hospital, Faculty of Veterinary Science, Mahidol University, Kanchanaburi Campus, Thailand (14°08'12.4"N 99°08'59.9"E or 14.136778, 99.149972). The research house has normal ventilation, which allows it to be exposed to light. This study was performed from September 2019 to August 2020. Each goat received 4-5 kg of dry Napier grass (*Cenchrus purpureum*) and 300 g of goat commercial concentrated feed per day. The concentrated feed contains the following chemical ingredients: moisture crude protein at least 14%, moisture crude fat at least 2.5%, moisture crude fiber less than 13%, humid at least 13%, and moisture crude carbohydrate with ash. Enough water in the basin was prepared for drinking throughout the day. All the goats ate normally and were healthy for the duration of this experiment.

Kanchanaburi province is located in the western region of Thailand. This area can be divided into 3 seasons: Summer (March to June), Rainy (July to October), and Winter (November to February) (Thai Meteorological Department, 2015). The Thai Meteorological Department in Kanchanaburi province provided the temperature, relative humidity, and daytime. Temperature humidity index (THI) was calculated in the following manner: $THI = 0.8 * \text{Temperature } (^{\circ}\text{C}) + \text{Relative humidity} * (\text{Temperature} - 14.4) + 46.4$ (Isnaini *et al.*, 2020). On the day of semen collection, the temperature and humidity changed in the research house during each semen collection were recorded and calculated to THI by the above formula. This data was done to confirm changes in the research house with climate data from the Thai Meteorological Department in Kanchanaburi province. However, recording temperature and humidity in a research house all year round is a limitation.

Semen Collection and Libido Score

In this study, goats were trained to collect semen using non-estrous dummy females and an artificial vagina. Semen quality had semen volume more than 0.2 mL, mass spermatozoa movement score more than 3, and percentage of spermatozoa motility at least 60 percent before being included in this study. From all eight male goats used in the observation, semen was collected monthly on the last Friday of the month from 9.00 am to 12.00 pm. After releasing the male to approach females, the libido score was assessed and scored from 0 (not interested in copulating more than 5 minutes) to 5 (copulating within 1 minute) (Frydrychova *et al.*, 2011).

Semen Quality Assessment

Semen quality consists of semen volume, semen sediment volume, seminal fluid volume, percentage of seminal fluid volume, spermatozoa concentration, percentage of spermatozoa motility, percentage of normal spermatozoa membrane integrity, and mass spermatozoa movement were evaluated after semen collection. Semen volume was measured using an autopipette. Seminal fluid was separated from spermatozoa by centrifugation (Thermo Scientific Heraeus Pico 17, Germany) at 5,000 rpm or about relative centrifugal force (RCF) 2.4 g for 15 minutes (Kimsakulvech *et al.*, 2018). In the case of incomplete separation, a second time of centrifugation at 8,000 rpm or RCF about 6.2 g for 10 minutes was done. Seminal fluid volume was measured using an autopipette and was calculated as a percentage of the seminal fluid volume. Semen sediment volume was calculated from semen volume minus seminal fluid volume. Spermatozoa concentration was estimated after diluting the semen sample in distilled water at 1:400 and counting the number of spermatozoa on a hemocytometer under a light microscope (Zeiss KF2-ICS, Germany) at 400× magnification (Hossain *et al.*, 2016).

The mass spermatozoa movement score, percentage of spermatozoa motility, and percentage of normal spermatozoa membrane integrity were grouped into spermatozoa functions. The mass spermatozoa movement was scored from 0 (immotile) to 5 (high), and the percentage of spermatozoa motility was evaluated from the percentage of spermatozoa moving forward under a light microscope at 400× magnification (Moreno-Avalos *et al.*, 2021). The percentage of normal spermatozoa membrane integrity was evaluated by diluting the semen with hypo-osmotic swelling solution (125 mOsm) at 1:400 and counting the number of bent-tail spermatozoa (normal membrane integrity) out of 200 total spermatozoa under a light microscope at 400× magnification (Zubair *et al.*, 2015).

Seminal Fluid Total Protein Analysis

Seminal fluid was stored at -20 °C before protein analysis. The total protein concentration of seminal fluid was quantified using the Bio-Rad Protein Assay (Bio-rad chemical, California, USA) according to the manufac-

turer's microplate assay protocol (Hashem & Eslami, 2018). Briefly, the dye reagent was diluted with deionized water at 1:4 and filtrated through a #1 Whatman filter paper. The bovine serum albumin (Bio basic Inc., Markham ON, Canada) as a standard protein was prepared in five dilutions in the linear range of 500 µg/mL to 5 µg/mL. Then the 10 µL of diluted standard and seminal fluid were added into a separate 96 well plate in duplicate. The 200 µL of diluted dye reagent was added to each standard and sample. The plate was incubated at an air-conditioned room temperature (25 °C) for 5 minutes. The absorbance was measured at 595 nm with the ELx808 ELISA microplate reader (BioTek, Winooski, Vermont 05404, USA).

Statistical Analysis

Climate factors (temperature, relative humidity, and THI) and daytime were compared among seasons by analysis of variance (ANOVA) and a post-hoc test with Duncan. Semen quality and seminal fluid protein were checked for normal distribution by the Shapiro-Wilk test and Kolmogorov-Smirnov ($p < 0.05$) before being compared among seasons by repeated measurement tests. The libido score, mass spermatozoa movement score, and percentage of motility were not found to be normally distributed. These parameters were further compared among seasons using the Kruskal-Wallis test

and followed by the Mann-Whitney test when statistical significance was found. The correlations between seasonal factors, semen quality and seminal fluid protein were evaluated by a simple Pearson correlation. All values were shown as the mean and standard deviation (SD). A statistically significant difference was performed at $p < 0.05$. Data were analyzed by using IBM SPSS Statistics for Windows, Version 18.0. Armonk, New York, USA.

RESULTS

In this study, the climate and daytime in Thailand changed with each season (Table 1) and month (Figure 1). The highest mean temperature was found in summer (May) and significantly higher ($p < 0.05$) than the lowest temperature found in winter (December). The highest mean relative humidity was found during the rainy season (September) and significantly higher ($p < 0.05$) than the lowest relative humidity found during the winter season (February). The highest THI was found in summer (May) and significantly higher ($p < 0.05$) than the lowest THI found in winter (December). At the above equator line, as in Thailand, the highest daytime was found in summer (June) and significantly higher ($p < 0.05$) than the lowest daytime found in winter (December), and the minimum daytime difference was only 1.37 hours. The seasonal and monthly changes

Table 1. Seasonal means (\pm SD) for temperature, relative humidity, temperature and humidity index (THI), and daytime outside research house during study period

Seasons	Temperature (°C)	Humidity (%)	THI	Daytime (hours)
Summer (Mar-Jun)	31.04 \pm 6.97 ^a	64.85 \pm 8.32 ^b	81.51 \pm 1.74 ^a	12.30 \pm 0.29 ^a
Rainy (Jul-Oct)	29.31 \pm 5.52 ^b	72.80 \pm 7.19 ^a	80.64 \pm 1.55 ^b	12.13 \pm 0.39 ^b
Winter (Nov-Feb)	27.39 \pm 6.93 ^c	62.25 \pm 7.52 ^c	76.41 \pm 2.77 ^c	11.28 \pm 0.09 ^c

Note: Means in the same column with different superscripts differ significantly ($p < 0.05$).

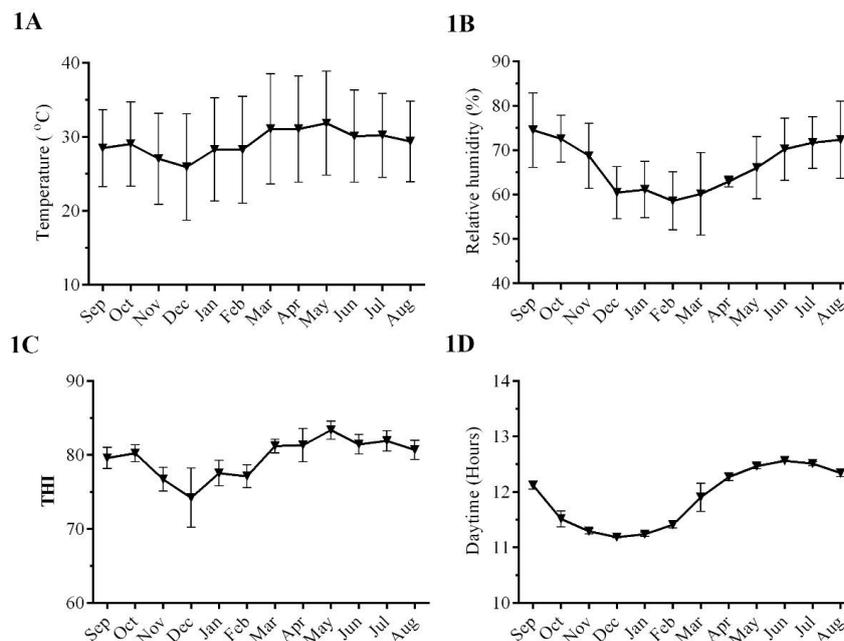


Figure 1. Monthly means (\pm SD) for temperature (1A), relative humidity (1B), temperature and humidity index or THI (1C), and daytime (1D) outside research house during study period

Table 2. Seasonal means (\pm SD) for temperature, relative humidity, and temperature and humidity index (THI) inside research house during study period

Seasons	Temperature ($^{\circ}$ C)	Relative humidity (%)	THI
Summer (Mar-Jun)	30.28 \pm 1.76 ^a	60.56 \pm 19.09 ^b	79.93 \pm 1.16 ^a
Rainy (Jul-Oct)	29.14 \pm 1.29 ^b	69.78 \pm 6.39 ^a	79.92 \pm 1.1 ^a
Winter (Nov-Feb)	29.11 \pm 0.95 ^b	51.31 \pm 4.5 ^c	77.41 \pm 1.2 ^b

Note: Means in the same column with different superscripts differ significantly ($p < 0.05$).

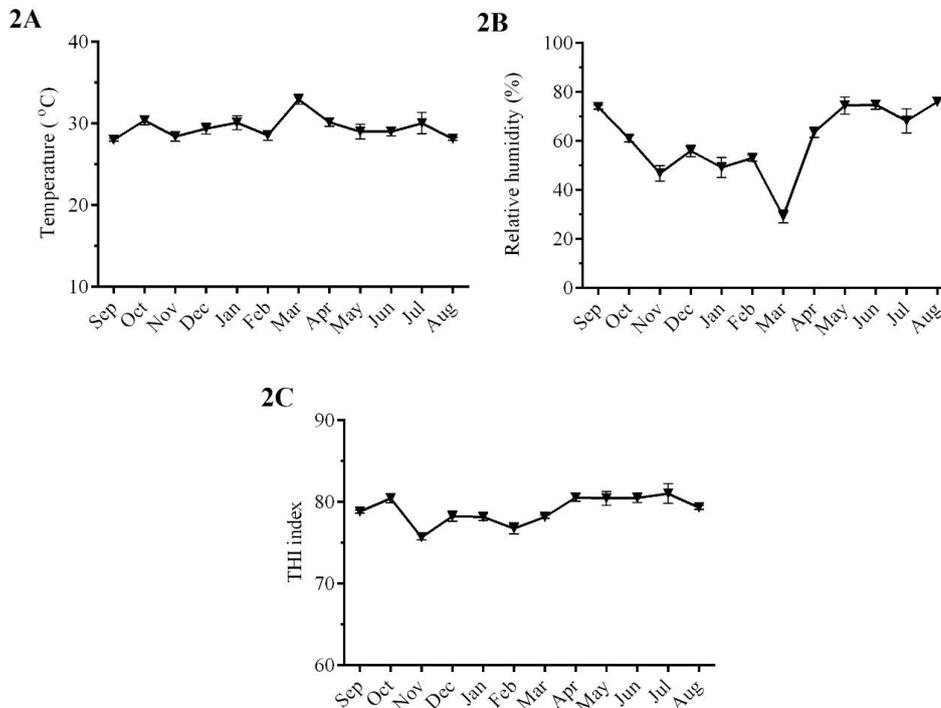


Figure 2. Monthly means (\pm SD) for temperature (2A), relative humidity (2B), and temperature and humidity index or THI (2C) inside research house during study period

in research house temperature, humidity, and THI were shown in Table 2 and Figure 2, respectively. The highest mean temperature was found in summer and significantly higher ($p < 0.05$) than in the other seasons. The highest mean humidity was found during the rainy season and significantly higher ($p < 0.05$) than other seasons. The duration of the summer season was shorter than the duration of the rainy season but longer than the duration of the winter season. The lowest THI was found during winter and significantly lower ($p < 0.05$) than those in the other seasons. The temperature and humidity in the research house correspond to climate data from the Thai Meteorological Department.

All of the experimental goats had normal sexual ability at the time of semen collection. The libido score was not different among seasons and months (Table 3 and Figure 3).

Some parameters of semen quality and seminal fluid protein levels varied by season (Table 3). Semen volume, seminal fluid volume, percentage of seminal fluid, and seminal fluid protein concentration in the rainy season were significantly higher ($p < 0.05$) than in the summer and winter. The highest semen volume was found in July, and the lowest was found in April. The highest volume of seminal fluid and the percentage

of seminal fluid volume were found in October, and the lowest was found in March (Figure 4A, 4B). The highest protein concentration in the seminal fluid was found in June, and the lowest was found in February (Figure 4B). The volume of semen sediment was relatively stable in all seasons and months (Table 3 and Figure 4A). By coincidence, spermatozoa concentrations in summer and winter were significantly higher than ($p < 0.05$) in the rainy season (Table 3). The highest spermatozoa concentration was found in October and the lowest was found

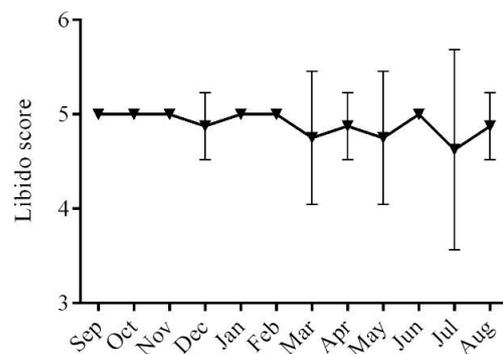


Figure 3. Monthly means (\pm SD) for goat libido score

Table 3. Seasonal means (± SD) of libido score, semen quality, seminal fluid volume, and seminal fluid protein in goat

Seasons	n	Libido score	Semen volume (mL)	Seminal fluid volume (mL)	Semen sediment volume (mL)	Percentage of seminal fluid volume	Total seminal fluid protein (mg/mL)	Mass movement score	Percentage of spermatozoa motility	Percentage of normal spermatozoa membrane integrity	Spermatozoa concentration (x10 ⁹ cells)	Total number of spermatozoa (x10 ⁹ cells)
Summer	32	4.84±0.51	0.54±0.23 ^b	0.30±0.17 ^b	0.24±0.1	53.17±11.59 ^b	40.54±14.23 ^b	4.47±0.80	80.63±12.49 ^a	66.17±19.30 ^a	7.63±2.43 ^a	3.99±1.74
Rainy	32	4.88±0.55	0.75±0.27 ^a	0.49±0.19 ^a	0.26±0.11	64.25±9.25 ^a	51.84±11.86 ^a	3.47±0.92	65.63±13.90 ^b	33.75±13.2 ^c	4.72±0.59 ^b	3.67±2.26
Winter	32	4.97±0.18	0.52±0.17 ^b	0.27±0.12 ^b	0.24±0.08	52.30±9.25 ^b	40.45±15.46 ^b	4.38±0.83	76.56±10.35 ^a	49.03±20.3 ^b	7.65±3.33 ^a	3.88±2.02

Note: Means in the same column with different superscripts differ significantly (p<0.05).

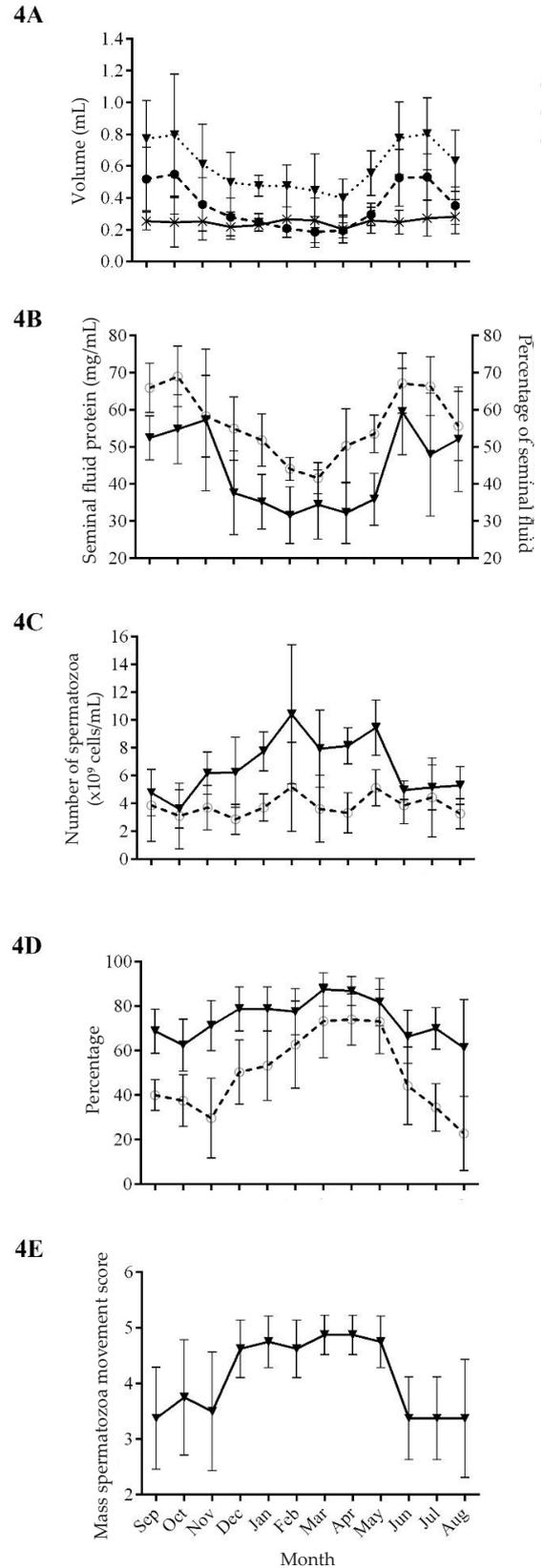


Figure 4. Monthly means (± SD) for semen volume (▼), seminal fluid volume (○), and semen sediment volume (x) (4A); percentage of seminal fluid volume (○) and total seminal fluid protein volume (▼) (4B); spermatozoa concentration (▼) and total number of spermatozoa (○) (4C); percentage of motility (▼) and normal spermatozoa membrane integrity (○) (4D); and mass spermatozoa movement score (4E) in goat.

in February (Figure 4C). The correlation coefficient between relative humidity and semen volume was highly significant ($p \leq 0.001$, $r = 0.894$). The change in the relative humidity could explain more than 70% of the change in semen volume ($r^2 = 0.78$). The correlation coefficients between semen volume, seminal fluid volume, percentage of seminal fluid, seminal fluid protein concentration, spermatozoa motility, spermatozoa concentration, and normal spermatozoa membrane were highly significant ($p < 0.001$). Changes in the percentage of seminal fluid could explain more than 90% ($r^2 = 0.99$) of the variation in the semen volume.

The lowest function of spermatozoa was found during the rainy season (Table 3). Spermatozoa motilities in summer and winter were significantly higher than in the rainy season. The highest spermatozoa motility was found in March and the lowest was found in August (Figure 4D). The percentage of normal spermatozoa membrane in the summer was significantly higher than in the winter and rainy seasons. For winter, the percentage of normal spermatozoa membrane was higher than in the rainy season. The highest percentage of normal spermatozoa membrane was found in April, and the lowest was found in August (Figure 4D). The score of mass spermatozoa movements in the summer and winter seasons tended to be higher than in the rainy season. The highest score of mass spermatozoa movement was found in April and May and the lowest score was found in June to September (Figure 4E). The correlation coefficient between relative seminal fluid protein and spermatozoa motility was highly significant ($p < 0.001$, $r = 0.865$). Changes in the seminal fluid protein could explain more than 70% of spermatozoa motility ($r^2 = 0.724$).

DISCUSSION

The habits of goats in the long dry season of tropical climate countries in this study had increased seminal fluid volume and seminal fluid protein concentration in the rainy season, which resulted in a drop in spermatozoa function, including mass spermatozoa movement score, percentage of spermatozoa motility, and percentage of normal spermatozoa membrane integrity. These results are different from those previously reported in other tropical countries (Aguiar *et al.*, 2013; Isnaini *et al.*, 2020).

The effects of season on semen quality in goats have been demonstrated in many studies (Arrebola & Abecia, 2017; Dias *et al.*, 2017). In the dry season of Indonesia, semen volume was higher than in the rainy season, while in the dry season of this study, semen volume was lower than in the rainy season. Semen concentration and spermatozoa motility improved during the rainy season in Indonesia and Brazil's Northeast (Aguiar *et al.*, 2013; Isnaini *et al.*, 2020). Contrary to this study, both parameters dropped in the rainy season. Seminal fluid volume did not have enough data on goats to compare among seasons. However, seminal fluid protein has been reported to be different among seasons (Aguiar *et al.*, 2013; Teixeira *et al.*, 2018). In the northeast of Brazil, seminal fluid was found to be higher in the dry season than in the rainy season (Aguiar *et al.*,

2013; Teixeira *et al.*, 2018). Nonetheless, in this study, seminal fluid volume and seminal fluid protein were higher in the rainy season than in the other seasons, which is different from previous reports in Brazil. Overall, goat semen quality improved during the rainy season in tropical climate countries near the equator line, such as Indonesia and Brazil's Northeast (Aguiar *et al.*, 2013; Isnaini *et al.*, 2020). In contrast with this study, goat semen quality greatly dropped in the rainy season in the tropical savanna climate of Kanchanaburi province, Thailand. Spermatozoa concentration and spermatozoa functions were the lowest for this season. These findings are comparable to those obtained in the Sahel region of Borno state, Nigeria (Maina *et al.*, 2006). Some climatic features and locations in Thailand and Nigeria are similar. Thailand and Nigeria are above the equator line and have a long dry period (about 8 months) than Brazil and Indonesia (da Silva, 2004; Isnaini *et al.*, 2020). Data on the seminal fluid volume had not enough to be used to compare at this time; however, seminal fluid protein had shown different among seasons (Teixeira *et al.*, 2018). In this study, the highest seminal fluid protein concentration was the highest in the rainy season. In northeast Brazil, the lowest seminal fluid protein concentration was found in high humid and cool seasons (Teixeira *et al.*, 2018). These results show that goats are well adapted to the long dry season. Likewise, according to the suggestions in previous study, genetics in native mixed breed goats in this study may be a part of adaptation to this climate (Maina *et al.*, 2006). Possibly, the major trigger for the changes of semen quality is a sudden change from a dry to a very wet climate or a humidity change. This trigger may induce a stress mechanism that influences hormone and reproductive changes (Díaz Pacheco *et al.*, 2018). The rainy season in tropical climates is a period for grass growth. This adaptation of male goats corresponds to an abundance of goat food, suitable for females giving birth and offspring (Pralomkarn & Boonsanit, 2012).

Seasons in tropical climate countries impact both semen quality and spermatozoa function. In this study, semen volume was correlated to relative humidity. Semen volume was increased in the rainy season, but the semen sediment volume and total spermatozoa were stable throughout the year. This result shows that spermatogenesis for producing sperm in the testis was not altered by the season. Semen consists of spermatozoa and seminal fluid (Leite-Browning, 2009). Also, the stability of semen sediment volume with a decrease in spermatozoa concentration indicated that an increase in semen volume was due to an increase in the seminal fluid volume. Currently, the increase in seminal fluid volume in the rainy season was not previously reported. However, the results of this study showed that an increase in seminal fluid protein was related to the lowest spermatozoa motility in the rainy season. According to previous studies, seasonal variation has influenced seminal fluid protein (Arrebola & Abecia, 2017; Teixeira *et al.*, 2018). Seminal fluid protein and composition changes may affect spermatozoa function (Umar *et al.*, 2018). The mechanism of increasing humidity in causing seminal fluid change has never been studied previously.

However, the growth of different plants differs between the rainy and dry seasons, which affects the proteins in the salivary digestion of these plants in goats. (Lamy *et al.*, 2012). Moreover, different climates in each season impact hormones and testicular echogenicity in goats (Samir *et al.*, 2020). Possibly, the change from the dry season to the wet season may induce stress followed by the changes in hormone secretion (Dias *et al.*, 2017), leading to the changes in seminal fluid protein and semen quality in goats.

Heat stress is a problem in the function of spermatozoa in small ruminants (Marai *et al.*, 2007). In this study, high ambient temperature during summer (around 37 °C) increased the function of spermatozoa compared to the other seasons. The high THI degraded semen quality in ruminants (Isnaini *et al.*, 2020; Llamas Luceño *et al.*, 2020; Sharma *et al.*, 2017). In this study, the high THI in the summer had semen quality, and most spermatozoa functioned similarly to the lowest THI in the winter season. In the rainy season, THI was lower than in the summer, but semen quality and spermatozoa function were lower than in the summer and winter. Goat semen quality has previously been reported to benefit from suitable THI (<72) (Ranjan *et al.*, 2020). In this study, the highest THI was found in May (83.3±1.2) and the lowest was found in December (74.3±4.0). These THI were higher than the comfortable THI suggestion for goats (Ranjan *et al.*, 2020). The THI in the tropical climates with long dry seasons was not related to goat semen quality changes. These results confirmed that goats could adapt to heat stress or resist heat, as was suggested in the previous report (Berihulay *et al.*, 2019). Noteworthy, high humidity has a greater effect on goat semen quality than temperature or THI.

Previously, seasonal breeding was reported in goats (Kafi *et al.*, 2004; Maina *et al.*, 2006). The key factor of seasonal differences in semen quality is photoperiod or daytime change. Goats are short-day breeders in some regions where the photoperiod affects behavior and semen quality (Arrebola & Abecia, 2017; Bedos *et al.*, 2016). In the country above the equator line, as in this study, the daytime throughout the year was slightly different or only 1.37 hours in the highest (between December and June). The difference in the mean daytime between the summer and rainy seasons was approximately 17 minutes and the increase between summer and winter was approximately 1 hour. In this study, male sex behavior was assessed using a libido score, which did not significantly differ between the seasons. For semen quality between summer and winter was similar. This study presented that the change in minimal daytime of country above equator line did not affect the sexual behavior of male goat and semen quality.

This study found that goats in Kanchanaburi province, Thailand, were well adapted to the long dry seasons with high temperatures and the differences did not affect the semen quality. Increasing protein contents of seminal fluid relates to the decreased semen quality in the rainy season. However, the mechanism of sudden increasing humidity in the rainy season on semen quality and seminal fluid protein should be further studied.

CONCLUSION

Wet seasons in tropical climates with long dry seasons increase seminal fluid volume and seminal fluid protein secretion, which relates to the decreased semen quality. Temperature, THI, and daytime changes throughout the year did not affect the semen quality of male goats and male sexual behavior.

CONFLICT OF INTEREST

This manuscript has no conflict of interest with any financial, personal, or other relationships with other people or organizations related to the material discussed.

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