Short Communication

Effect of heat stress on reproductive performance of an imported dorper ram: a case study in Thailand

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Abstract

The aim of this study was to observe the possible influence of seasonal and environmental conditions on ejaculated semen qualities and male reproductive traits in an imported ram under tropical conditions. A Dorper ram was imported from South Africa and raised in Nakhon Pathom province. Semen was collected monthly by an artificial vagina over a period of one year. Semen picture and other parameters were evaluated in each sample. Climatic data (temperature, relative humidity and day length) were provided by the Meteorological Department of the Ministry of Communications of Thailand. Results showed that semen characteristics (volume, concentration, mass motility, motility and percentage of normal spermatozoa) of this ram significantly varied according to temperature, temperature-humidity index (THI) and day length, while scrotal circumference was only significantly associated with day length. Characteristic peak of the Dorper ram's reproductive traits was shown during the cool season from December to March.

Keywords: heat stress, imported ram, semen characteristics, THI, day length, tropical area

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Introduction

The production of high semen quality from a high genetic potential male is a key to success in breeding improvement through cross-breeding. Therefore, the importation of male animals of high genetic merit is a practical action to expand an animal herd. Howerver, one key factor that affects semen production in males is heat stress (Hansen, 2009). In tropical countries, poor reproductive performances, including male sexual behavior, semen production and quality, have been observed to decline in many species (Kumi-Diaka et al., 1981; Ahmed et al., 1997; Suriyasomboon et al., 2005; Nichi et al., 2006; Koonjaenak et al., 2007) including in male sheep (El-Darawany, 1999; Cárdenas-Gallegos et al., 2015). In bovine raised in a tropical region, the extended period of humid temperatures and longer day lengths was found to affect reproduction (Bouraoui et al., 2002; Somparn et al., 2004; Suadsong et al., 2008); this was also found in swine reproduction (Kunavongkrit and Heard, 2000), as semen volume and quality reduced significantly in summer. It has also been found that a high temperature and high relative humidity have a negative effect on goat's reproduction as these conditions reduce semen volume and sperm concentration. However, under tropical conditions with high ambient temperature and humidity, semen production of rams is still unclear. Therefore, the present study performed serial measurement over 12 months of semen characteristics and scrotal circumference in one imported Dorper ram to determine if male reproductive performance is possibly affected by climatic conditions in Thailand.

Materials and Methods

Animal: A Dorper ram born in South Africa in September 2011 was purchased and brought to the Large Animal Hospital, Faculty of Veterinary Science, Chulalongkorn University in Nakhon Pathom province (Central Thailand) in October 2012. After arrival, the ram appeared physically normal and received general health examination, including physical, complete blood count (CBC) and fecal examination, and was tested for brucellosis, bluetongue and paratuberculosis. The ram was then housed in a semi-intensive protective system with clinical veterinary care and fed individually on concentrate (12% crude protein) and grass *ad libitum*. Drinking water and mineral block were made available at all times. The ram was two years old and about 90 kg at the onset time of collection.

Measurement of scrotal circumference, semen collection and evaluation: Scrotal circumference was measured by the same person once a month over 12 months by applying a tape measure at the greatest width of the testicular scrotum while the ram was in a standing position. Semen was collected using the artificial vagina (AV) technique described by Moore (1985). The semen was collected once a month from July 2013 to June 2014. Semen volume, consistency and color were macroscopically evaluated at instant post-ejaculation time. The semen volume was assessed from a graduated collecting tube. The color and consistency of the evaluated semen were classified as creamy, milky, opaque or watery.

pH of the semen was measured with a pH-indicator paper (Neutralit®, Merck, Germany). Mass motility and motility of the ejaculated semen were evaluated under a phase contrast microscope at 40X and 100X magnification, respectively (Olympus® microscope model, Shinjuku, Japan), using the numerical scale of 0 to 4 mass motility (Islam et al., 2006). The sperm concentration was diluted to 1:400 and determined by a hemocytometer (Neuber®, Town, Germany). Stained semen smear was performed by diluting mixed semen with eosin-aniline blue stains to examine sperm morphology.

Climatic data (temperature, relative humidity and day length) were provided by the Meteorological Department of the Ministry of Communications of Thailand. Data were collected from meteorological stations in Nakhon Pathom, Thailand for period of one year, from July 2013 to June 2014. Data were collected at 4:00 am and 4:00 pm to represent morning and evening climates.

Temperature-humidity index (THI) was calculated at each time point using the equation developed by Spiers et al. (2004), a dimensionless static, which is expressed as:

$$THI = (((1.8 \times T) + 32) - (0.55 - (0.0055 \times H))) \times (((1.8 \times T) + 32) - 58)$$

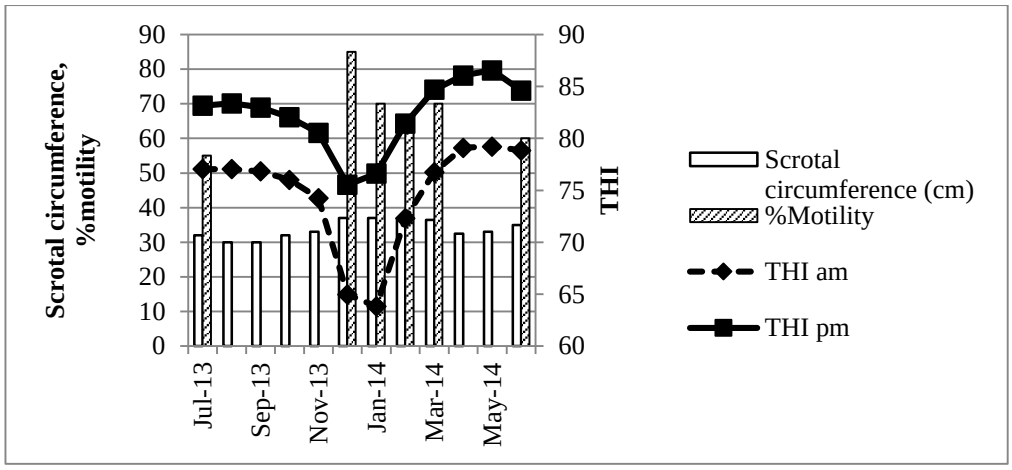
where T is the mean value of temperature in ^{o}C and H is the % of mean value of relative humidity.

Data were shown in descriptive statistics. The Spearman's rank correlation was used to determine significance of correlations between semen characteristics and climatic data.

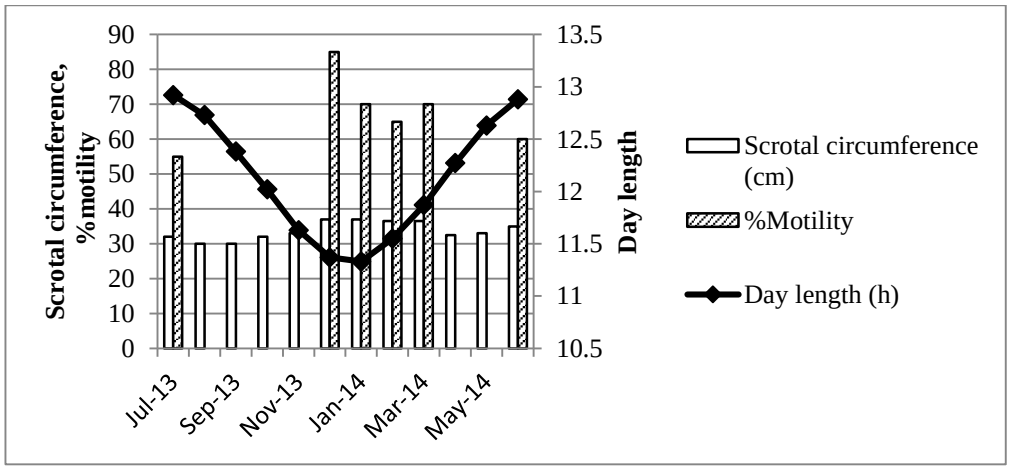
Results and Discussion

The variation of temperature, day length and THI at the experiment site are shown in Figure 1. It was found that the scrotal size was significantly larger in months with lowest temperature, from December to March, when compared to in the other months (mean testicular 36.8 v 32.2 cm, respectively). In this case, the scrotal circumference significantly correlated with the number of ejaculated spermatozoa (Spearman's $\rho=0.78$, $p<0.05$), mass motility (Spearman's $\rho=0.80$, $p<0.01$), motility (Spearman's $\rho=0.84$, $p<0.01$), percentage of normal morphology (Spearman's $\rho=0.89$, $p<0.01$), and semen volume (Spearman's $\rho=0.58$, $p<0.05$). It was found that the heat stress had a significant effect on semen quality, as while the animal still showed a libido, watery ejaculated semen with azoospermia was collected. Due to the fact that the complete duration of a ram's spermatogenic cycle is 47-48 days (Zeng et al., 2006), the initiation of heat stress period at the arrival of this imported ram (July 2013) and the beginning of summer (Febuary 2014) seemed to affect semen quality in the next 1.5-2 months. This observation corresponds to Irahim (1997), who reported that the highest peak temperature during summer had a negative effect on semen quality in rams. Similarly, Karagiannidis et al. (2000), studying Chios and Friesian rams, reported that the positive correlation between high ambient temperatures and increasing day length during summer months caused

a decrease in ejaculated semen quality. The results of their studies agree with this report; the climatic parameters (temperature, THI, and day length) strongly negatively correlated with the ejaculated semen quality (number of spermatozoa, motility, percentage of normal morphology: $p<0.01$) while the scrotal circumference was only significantly asscociated with day length ($p<0.01$). The semen quality did improve when the temperature started to decrease in October, and the animal was able to reproduce sperm of a higher quality beginning in December. In contrast with the present study, acceptable semen quailty was still reproduced throughout the year in goats although seasonal conditions had a pronounced effect upon ejaculated semen traits in this species (Anakkul, 2012). Moreover, higher quality semen for insemination or freezing was found to take place more as the goat appeared to adapt better to a hot climate when compared with sheep and cattle (Coop, 1982; Valez-Nauer et al., 1982).



A



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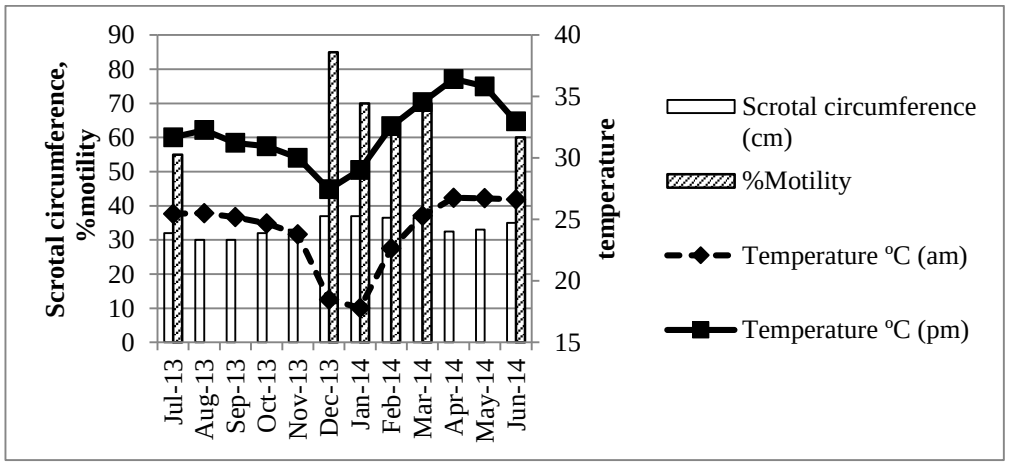

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Figure 1 Climatic data, percentage of sperm motility and testicular circumference of the imported Dorper ram in each month during the study period (July 2013-June 2014)

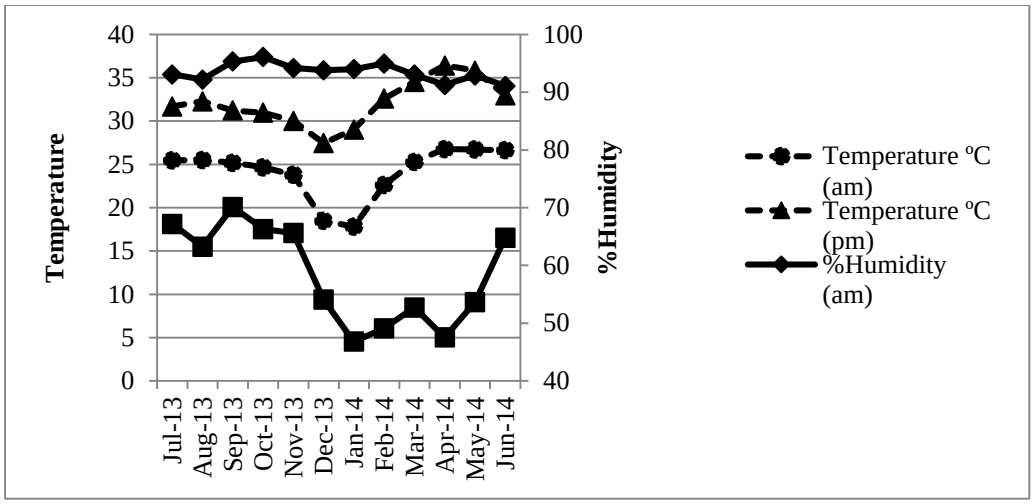

Figure 2 Temperature and percentage of humidity in each month during the study period (July 2013-June 2014).

THI is an indicator used to measure the effect of heat stress on ruminant production in tropical or subtropical areas (Bouraoui et al., 2002). In this study's region, THI had mostly mild stress (72-78) in the morning and medium stress (79-88) in the afternoon (see also Figure 1A). This study found that male reproductive performance (ejaculated semen quality and scrotal size) improved when THI value decreased from 74 to 64 and 80 to 75 in am and pm, respectively. El-Darawany (1999) reported that rams suffered from severe heat stress when THI was above 84.3. In addition, the result of decrease in scrotal and testicular circumferences during high ambient temperature agrees with that of Yarney et al. (1990). Together with our findings, scrotal circumference, testicular size and tone are great indicators of spermatogenesis and sperm production in rams as well as THI index.

Table 1 Semen characteristics (semen volume, pH, total number of sperm, mass motility, % motility and % normal sperm morphology of head and tail) of the imported Dorper ram in each month during 1-year study period (July 2013-June 2014)

Month	semen volume (ml)	pH	Total number of sperm (×10⁹)	mass motility[1]	Motility (%)	Normal Head (%)	Normal Tail (%)
Jul-13	1.75	7.5	1.3	2	55	47.8	80.2
Aug-13	2	7	1.1	0	0	52.2	72
Sep-13	1.5	7.5	NA	0	0	ND	ND
Oct-13	1	8	NA	0	0	ND	ND
Nov-13	1.8	7	NA	0	0	ND	ND
Dec-13	2.5	7	1.8	3	85	99.4	95.4
Jan-14	3.8	7.5	6.5	3	70	99.2	88.5
Feb-14	3.5	7.5	1.8	2.5	65	98.8	85
Mar-14	1	7	1.5	3.5	70	98.4	87.5
Apr-14	2	7	NA	0	0	ND	ND
May-14	2.5	8	0.2	0	0	46	72
Jun-14	3	7.5	1.0	2	60	54.2	77.5

[1] Based on a scale of 0 to 4; 4 = best
NA = Not applicable because the number of sperm was less than 10 million
ND = Not detected

Table 2 Correction between climatic data and reproductive characteristics in the Dorper ram

Reproductive characteristics	Temperature		THI		Day length	
	Correlation coefficient	p value	Correlation coefficient	p value	Correlation coefficient	p value
Total number of sperm	-0.994	<0.001*	-0.994	<0.001*	-0.778	0.023*
Motility (%)	-0.535	0.073	-0.587	0.045*	-0.501	0.097
Normal head (%)	-0.881	0.004*	-0.881	0.004*	-0.810	0.015*
Normal tail (%)	-0.910	0.002*	-0.910	0.002*	-0.731	0.039*
Scrotal circumference	-0.508	0.092	-0.554	0.062	-0.698	0.012*

Data were analyzed using Spearman's rank order correlation.

Furthermore, the percentage of sperm abnormality (teratozoospermia) increased with higher temperatures and humidity. The most common morphological abnormalities of sperm observed were headless, tailless, microcephalic, spermatid and coiled tail around the head (see also Figure 3). Percentage of

abnormal sperm morphology was higher during the non-breeding season (spring and summer), while the breeding season was a transferrable period with normal sperm (Mickelsen et al., 1981; Karagiannidis et al., 2000). It should be noted that semen production and sperm output recovered when the heat stress disappeared during the cool season. This means that the semen production of the imported ram could recover when the climatic conditions returned to normal range.

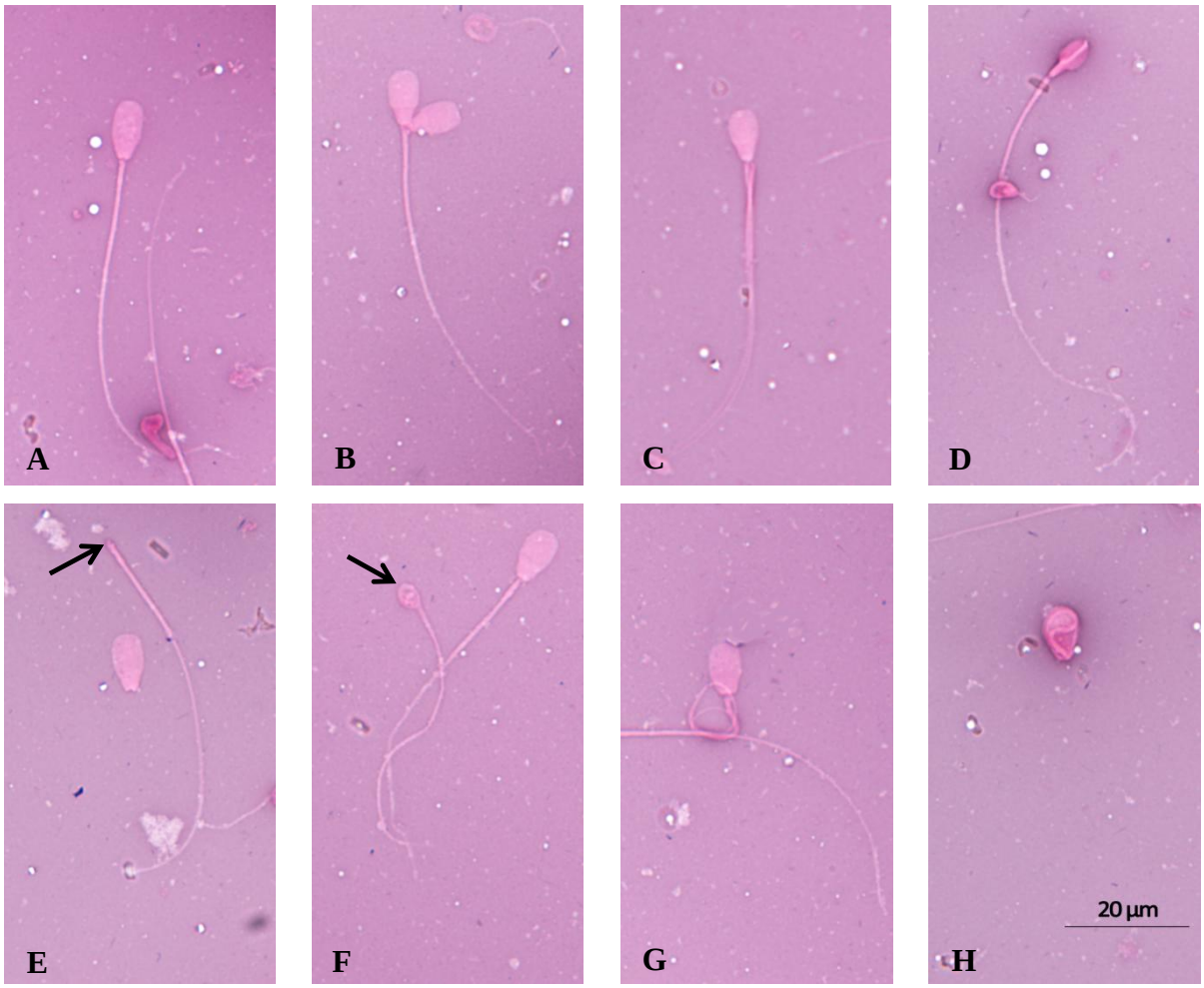

Figure 3 Microscopic view of ram sperm stained with Eosin-aniline blue stain (magnification: 1000X). Normal spermatozoa (A), Bicephalic (B), Biflagella (C), Microcephalic (D), Headless tail (arrow) and tailless head (E), Spermatid (arrow, F), Tightly coiled tail (G), and Coiled tail around the head spermatozoa (H).

In conclusion, from the serial observation of semen quality and scrotal circumference over 12 months, the imported ram showed higher performances with acceptable sperm characteristics during the cool season (from December to March). Moreover, good management strategies for decreasing heat stress such as applying shading, cooling or air conditioning systems might help improve the semen quality of imported ram raised in a tropical area.

Acknowledgements

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บทคัดย่อ

รายงานการศึกษา: ผลกระทบของภาวะเครียดจากความร้อนต่อสมรรถภาพการสืบพันธุ์ของพ่อแกะพันธุ์ดอร์เปอร์นำเข้า

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วัตถุประสงค์ของการศึกษาครั้งนี้เพื่อศึกษาโอกาสที่ฤดูกาลและสิ่งแวดล้อมจะมีผลกระทบต่อคุณภาพและคุณลักษณะของน้ำเชื้อของพ่อพันธุ์แกะนำเข้า ทำการนำพ่อแกะพันธุ์ดอร์เปอร์เข้ามาจากประเทศแอฟริกาใต้และนำมาเลี้ยงที่จังหวัดนครปฐม ทำการเก็บน้ำเชื้อเพื่อตรวจคุณภาพทุกเดือนเป็นระยะเวลาหนึ่งปี ส่วนข้อมูลสภาพภูมิอากาศได้รับการสนับสนุนจากกรมอุตุนิยมวิทยา การศึกษาพบว่าคุณภาพน้ำเชื้อ ได้แก่ ปริมาตร ความเข้มข้น การเคลื่อนไหวรายหมู่ของตัวอสุจิ การเคลื่อนไหวรายตัว และเปอร์เซนต์รูปร่างปกติของตัวอสุจิของพ่อแกะผันแปรตามอุณหภูมิ ความชื้น และปริมาณแสง และพบว่าขนาดวงรอบของถุงหุ้มอัณฑะมีความสัมพันธ์กับปริมาณแสง โดยพ่อแกะมีคุณลักษณะทางระบบสืบพันธุ์สูงสุดในระหว่างเดือนธันวาคมถึงมีนาคม

คำสำคัญ: *ภาวะเครียดจากความร้อน พ่อแกะนำเข้า คุณลักษณะของน้ำเชื้อ ค่าดัชนีอุณหภูมิและความร้อนชื้น ปริมาณแสง เขตร้อนชื้น*

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