*Original Article*

# Season and Breed Effects on Sperm Production in PRRS Free Boars

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## *Abstract*

This study was carried out to investigate sperm production in tropical area of boar stud which has been free from porcine reproductive and respiratory syndrome (PRRS) more than ten years and kept in evaporative cooling system, in relation to season and breed influence. Semen production data from 19,966 ejaculates of 517 boars (164 Duroc, 31 Pietrain, 268 Landrace x Yorkshire: LY, 54 Pietrain x Duroc: PD) were collected. Semen parameters; volume (ml), sperm concentration ($x10^6$ sperm/ml) and total number of sperm per ejaculate ($x10^9$ sperm/ejaculate) were evaluated. The semen production was shown by month and group in relation to season as summer (Mar-Jun), rainy season (Jul-Oct) and winter (Nov-Feb). On average, the semen volume, concentration, and total sperm per ejaculate were 249.7±97.5 ml, 335.7±95.9$x10^6$ sperm/ml and 78.9±28.4$x10^9$ sperm/ejaculate, respectively. For the effect of season, the total number of sperm per ejaculate in winter was higher than a period in rainy season (Aug-Oct) ($p< 0.05$) while the concentration in early winter (Nov and Dec) was lower than summer and a month in rainy (Jul) ($p< 0.05$). Effect of breed, the total sperm production of LY crossbred boar (88.2±27.2 x $10^9$ sperm/ejaculate) was higher than purebred Duroc boar (60.2±21.9 x $10^9$ sperm/ejaculate, $p= 0.01$) and purebred Pietrain boar (76.5±21.8$x10^9$ sperm/ejaculate, $p= 0.03$). The seasonal variation effect was most pronounced in purebred Duroc and Pietrain boars rather than LY and PD crossbred boars. The fertility data using Duroc semen (total number of piglets born and born alive) tended to increase during Mar-Jun and Oct-Dec. The lowest total number of piglets born and born alive were shown in Oct ($p< 0.05$). It can be concluded that season and breed influencing the sperm production was found in boars kept in EVAP with free PRRS in Thailand.

**Keywords:** boar, breed, free PRRS, season, semen

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# บทคัดย่อ

**ผลของฤดูกาลและพันธุ์ต่อประสิทธิภาพน้ำเชื้อของพ่อพันธุ์สุกรที่มีสถานะปลอดโรค PRRS**

*ชาญยุทธ ตรีทิพย์สกุล นัทธี อ่ำอินทร์ เผด็จ ธรรมรักษ์ มงคล เตชะกำพุ**

ศึกษาผลกระทบของฤดูกาลและพันธุ์ในเขตพื้นที่ร้อนชื้นต่อการผลิตน้ำเชื้อของฝูงสุกรพ่อพันธุ์ ที่มีสถานะปลอดต่อโรคกลุ่มอาการทางเดินหายใจและระบบสืบพันธุ์ (PRRS) มาตลอดเป็นระยะเวลามากกว่า 10 ปี ในโรงเรือนระบบปิด EVAP จากข้อมูลการตรวจคุณภาพน้ำเชื้อ 19,966 ครั้งของสุกรพ่อพันธุ์ จำนวน 517 ตัว แบ่งเป็นพ่อสุกรสายพันธุ์ดูร็อค จำนวน 164 ตัว สายพันธุ์เปียแทรง จำนวน 31 ตัว สายพันธุ์ผสมแลนด์เรซกับยอร์คเชียร์ (LY) จำนวน 268 ตัวและสายพันธุ์ผสมเปียแทรงกับดูร็อค (PD) จำนวน 54 ตัว แสดงผลการตรวจคุณภาพน้ำเชื้อวัดจากปริมาตร (มล.) ความเข้มข้น (x $10^6$ sperm/มล.) และจำนวนอสุจิทั้งหมดต่อการหลั่งต่อครั้ง (x $10^9$ sperm/มล.) ตามรายเดือนและจัดกลุ่มเดือนตามฤดูกาล (ฤดูร้อน, มีนาคมถึงมิถุนายน: ฤดูฝน, กรกฎาคมถึงตุลาคม: ฤดูหนาว, พฤศจิกายนถึงกุมภาพันธ์) ผลค่าเฉลี่ยของการตรวจวัดปริมาตรน้ำเชื้อมีค่าเท่ากับ 249±97.5 มล. ค่าความเข้มข้นเท่ากับ 335.7±95.9 x $10^6$ ตัวอสุจิ/มล. และจำนวนอสุจิทั้งหมดต่อการหลั่งต่อครั้งเท่ากับ 78.9±28.4 x $10^9$ ตัวอสุจิ/มล. เมื่อเปรียบเทียบตามช่วงฤดูกาลพบว่าจำนวนอสุจิทั้งหมดต่อการหลั่งต่อครั้งในฤดูหนาวนั้นมีค่าสูงกว่าเดือนสิงหาคมถึงตุลาคมในฤดูฝน ($p< 0.05$) ในขณะที่ค่าความเข้มข้นในช่วงเดือนต้นฤดูหนาว (พฤศจิกายนและธันวาคม) มีค่าต่ำกว่าช่วงฤดูร้อนและช่วงฤดูฝนในเดือนกรกฎาคม ($p< 0.05$) และจากผลของพันธุ์พบว่าค่าจำนวนอสุจิทั้งหมดต่อการหลั่งต่อครั้งของพ่อพันธุ์ผสม LY (88.2±27.2) มีค่าสูงกว่าสายพันธุ์แท้ดูร็อค (60.2±21.9; $p= 0.01$) และเปียแทรง (76.5±21.8; $p= 0.03$) (x $10^9$ sperm/มล.) แสดงว่าพันธุ์และฤดูกาลมีผลกระทบกับพ่อสุกรพันธุ์แท้ดูร็อคและเปียแทรงต่อการผลิตน้ำเชื้อมากกว่าในพ่อสุกรพันธุ์ผสม LY และ PD ซึ่งข้อมูลความสมบูรณ์พันธุ์ (จำนวนลูกสุกรเกิดทั้งหมดและลูกสุกรมีชีวิต) ที่ใช้น้ำเชื้อจากสายพันธุ์ดูร็อคระหว่างเดือนมีนาคมถึงมิถุนายน และตุลาคมถึงธันวาคมมีแนวโน้มเพิ่มขึ้น และพบว่าจำนวนลูกสุกรเกิดทั้งหมดและลูกสุกรมีชีวิตมีค่าต่ำสุดในเดือนตุลาคม ($p< 0.05$) สรุปได้ว่าฤดูกาลและสายพันธุ์มีผลกระทบต่อการผลิตน้ำเชื้อของพ่อพันธุ์สุกรที่เลี้ยงในโรงเรือนระบบปิด EVAP และมีสถานะปลอดโรค

**คำสำคัญ:** สุกรพ่อพันธุ์ พันธุ์ สถานะปลอดโรค PRRS ฤดูกาล น้ำเชื้อ

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## *Introduction*

Sperm production of boars is affected by several factors such as breed, season, nutrition and housing (Ciereszko et al., 2000; Kunavongkrit et al., 2005; Huang et al., 2010). It was found that the semen production of purebred and crossbred boar including breed lines was different (Sonderman and Luebbe, 2008). Thailand is situated in tropical zone where the temperature is normally above $30^0$C for several months of the year, Reduction in pig production and reproductive performance are significantly observed during hot periods. As a result, evaporative cooling system is normally used for housing in Thailand to ensure good semen production. This system helps to reduce the seasonal variation of the sperm production in Duroc boars (Suriyasomboon et al., 2004). Moreover, the semen must be produced from disease-free boar to prevent disease transmission via semen. It has been known that porcine reproductive and respiratory syndrome virus (PRRSV) infection is a very important disease of swine due to its transmission from infected boar to several sows which subsequently affects reproductive performances (Guérin and Pozzi, 2005). The establishment of isolated free PRRS boar station is required for semen distribution to sow herds, which has been successfully done in Thailand. The objectives of the present study were to investigate the sperm production of boar stud which has been free from PRRS in Thailand in relation to breed, seasonal influences and fertility data in a few sow herds.

## *Materials and Methods*

***Data of semen production:*** This study was based on semen production collected from a central isolated boar station in the eastern part of Thailand during the period of January 2006 until December 2009. This station provided semen for AI to three breeder farms (n= 10,000 sows) all year round. A total of 19,966 ejaculates were collected from 517 boars with two purebreds (Duroc, n= 164; Pietrain, n= 31) and two crossbred boars (Landrace x Yorkshire: LY, n= 268; Pietrain x Duroc: PD, n= 54). All boars were trained and identified as proven sires from their fertility data of artificial insemination in PRRS free herds. The seronegative of PRRS was tested with the LAB

(ISO/IEC17025:2005) and had been operated for at least 10 years before the start of the study.

***Semen collection and evaluation:*** The ejaculates were collected routinely using the gloved-hand method and evaluated macroscopically and microscopically as subjective motility assessment. The gel free semen parameters including volume (ml) measured by weight, sperm concentration (x $10^6$ sperm/ml) measured by Spermacue® (Minitube, Germany) and total number of sperm per ejaculates (x $10^9$ sperm/ejaculate) calculated by multiplying ejaculating volume and sperm concentration were evaluated. To relate to seasonal effect, the data of daily temperature and humidity were recorded during the studying period.

***General herd management and serology monitoring:*** Each boar was kept in individual pens (9 $m^2$/boar) and was fed on 2.2-3.0 kg/day of commercial feed containing 14-18% crude protein. The boars had access to water *ad libitum* via nipple. The young replacement boars produced from a GGP farm classified as free PRRS status were penned in the quarantine area at least 2 weeks before being trained. The clinical disease and serial blood monitoring were observed during quarantine period. After absolutely free PRRS evidence, all replaced boars were trained and the semen was collected at least twice for evaluation for volume, concentration and motility. To maintain PRRS negative status, all boars were routinely tested for seronegative (S/P ratio < 0.2) by ELISA (HerdChek-PRRS®; IDEXX, Laboratories Inc., Westbrook, MA, USA) and PCR from Animal Health and Technical Service Office.

***Season, temperature and humidity:*** *Semen* production was calculated in relation to season as summer (March to June), rainy (Jul-Oct) and winter (Nov-Feb). Temperature and humidity were recorded once a day. The Max-Min thermometer which was hung at the center of the housing about 170 cm above the floor was used to record the temperature inside EVAP. Temperature was record every day about 7-8 am, after each recording the device was set to measure a new figure for the next day. The percentage of humidity in EVAP was recorded as the average humidity by using a digital device (TEMP1000®; Italy) which had 2 hygro-sensors located about 20 meters far from each other along the front and back of the housing. The outside temperature and humidity were recorded from The Eastern Part of Thai Meterological Department, Thailand. The two temperature variables were defined as the number of hot days per month (maximum temperature > $25^0$C) and the number of optimum days per month (maximum temperature $\leq$ $25^0$C) (Auvigne et al., 2010).

***Fertility data collection:*** To find the relationship between sperm production and fertility, data were collected from three breeder farms located around this boar stud not more than 50 km. All of them were EVAP system housings. Semen usage, 62,404 of fertility data of Duroc boar semen from the boar stud during January 2006 until December 2009, were included in this study. This fertility data comprised total piglet born and born alive.

***Statistical analysis:*** The sperm production data were analyzed using general linear mixed model procedure (MIXED) of SAS version 9.0 (SAS®, NC, USA). The models included boar breeds, year and month in which the semen was collected as fixed effect and included boar identity tested within breed as random effect. Least-squared means were obtained and compared among breeds using Tukey-Kramer test. The total number of piglets born were analyzed using general linear model procedure (GLM) to compare among the month of farrowing data from each farm during 2006-2009. $p< 0.05$ was regarded as a significant difference.

## *Results*

On the average, semen production resulted as descriptive data in each breed is presented in Table 1. Across the breeds, the average semen volume, concentration, and total sperm per ejaculate were 249.7±97.5 ml, 335.7±95.9x$10^6$ sperm/ml and 78.9±28.4x $10^9$ sperm/ejaculate, respectively. Duroc boar presented the lowest mean value of volume, 170.0±67.8 ml, but possessed the highest concentration. For the overall sperm output (78.9±28.4 sperm/ejaculate), LY had the highest mean value of total sperm per ejaculate (88.2±27.2 x $10^9$ sperm/ejaculate).

Temperature ($^0$C) and humidity (%RH) between outside and inside EVAP system are shown in Fig 1a. EVAP system enables the outside temperature to be lowered about 4-6$^0$C while the humidity was increased due to its spraying and

**Table 1** Means, standard deviation (SD) and range of sperm production in Duroc (D), Pietrain (P), Landrace x Yorkshire (LY) and Pietrain x Duroc (PD) boars kept in EVAP

| Variables | Breed | N | Mean±SD | Range |
|---|---|---|---|---|
| Volume (ml) | D | 5,763 | 170.0±67.8 | 50-497 |
| | P | 749 | 249.5±72.2 | 65-486 |
| | LY | 11,729 | 293.8±86.0 | 50-500 |
| | PD | 1,725 | 250.7±76.1 | 58-500 |
| | All | 19,966 | 249.7±97.5 | 50-500 |
| Concentration (x $10^6$sperm/ml) | D | 5,253 | 381.6±88.9 | 75-600 |
| | P | 659 | 318.3±91.2 | 97-600 |
| | LY | 11,232 | 310.0±89.6 | 80-600 |
| | PD | 1,283 | 381.1±93.3 | 105-600 |
| | All | 18,427 | 335.7±95.9 | 75-600 |
| Total sperm per ejaculate (x $10^9$ sperm) | D | 5,229 | 60.2±21.9 | 12-255 |
| | P | 654 | 76.5±21.8 | 19-230 |
| | LY | 11,058 | 88.2±27.2 | 17-284 |
| | PD | 1,279 | 76.4±26.5 | 15-203 |
| | All | 18,220 | 78.9±28.4 | 12-284 |

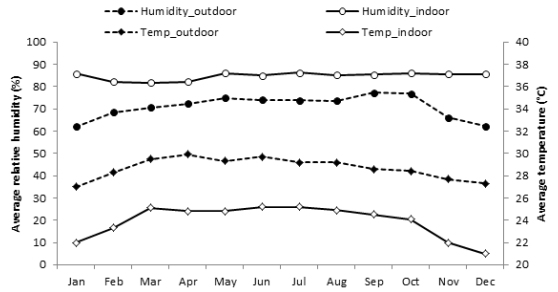


**Figure 1a** Average temperature and humidity outside and inside the boar stud equipped with evaporative cooling system (Summer, Mar-Jun; Rainy, Jul-Oct; and Winter, Nov-Feb)

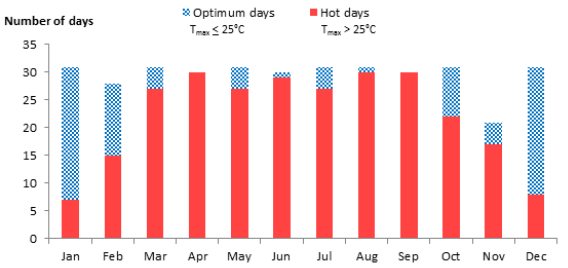


**Figure 1b** Number of hot days (maximum temperature >25$^0$C) with the month of inside temperature of the boar stud equipped with evaporative cooling system (Summer, Mar-Jun; Rainy, Jul-Oct; and Winter, Nov-Feb)

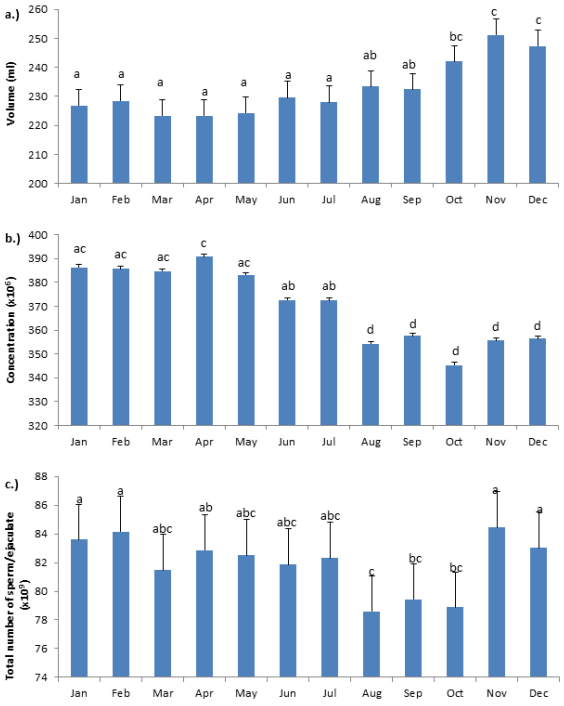


**Figure 2** Semen production (a. volume, b. concentration and c. total number of sperm) compared by months with $^{a,b,c}$ as different superscripts indicating significant difference ($p$< 0.05) (Summer, Mar-Jun; Rainy, Jul-Oct; and Winter, Nov-Feb)

cooling system. The number of days with a maximum temperature of >25$^0$C were classified as hot days; and the days with a maximum temperature of ≤ 25$^0$C as optimum days (Fig 1b). It was revealed that the hot days were more than twenty days a month in Mar-Jun

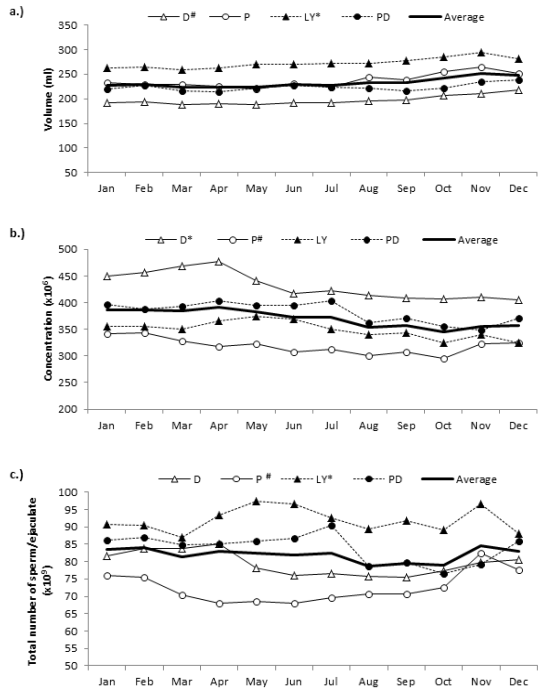


**Figure 3** Semen production (a. volume, b. concentration and c. total number of sperm) in Duroc (D), Pietrain (P), Landrace x Yorkshire (LY) and Pietrain x Duroc (PD) boars by months with $^{*, \#}$ as different superscripts in each legend indicating significant difference ($p$< 0.05) as the highest and the lowest in each parameter among breed, respectively

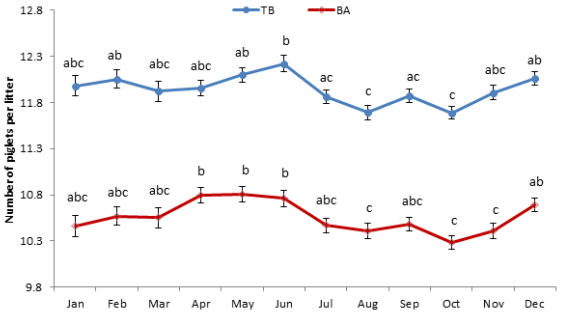


**Figure 4** Least-squared means and standard error of total piglets born by months with $^{a,b,c}$ as different superscripts in each line indicating significant difference ($p$< 0.05) (Summer, Mar-Jun; Rainy, Jul-Oct; and Winter, Nov-Feb)

(summer) and Jul-Oct (rainy) and all the days in April and September were hot days.

Monthly sperm production is presented in Fig 2 ((a) volume, (b) concentration and (c) total sperm per ejaculate). The volume was obviously significantly different in early winter (Nov and Dec) ($p$< 0.05). On the contrary, the obviously significant difference of concentration was found during middle rainy to early winter (Aug to Dec) ($p$< 0.05).

Accordingly, the total number per ejaculate during Nov to Dec had no difference ($p$> 0.05) but during Aug to Oct had a significant difference ($p$< 0.05).

The sperm production in each breed is presented in Fig 3 ((a) volume, (b) concentration and

(c) total sperm per ejaculate) in each average parameter production. Across the months, LY had the highest volume and total sperm production ($p< 0.05$) throughout the year, while Pietrain possessed the lowest concentration and total sperm production ($p< 0.05$). Duroc produced the lowest volume but produced the highest concentration all year round ($p< 0.05$). The total sperm production of LY crossbred boar was higher than Duroc ($p= 0.01$) and Pietrain ($p= 0.03$).

The fertility data, 2006-2009, was recorded from sow herds by using Duroc semen, as shown in Fig 4. The total number of both piglets born and born alive tended to increase during summer (Mar-Jun) and Oct-Dec. Meanwhile, in June to August, the total number of piglets born and born alive tended to decrease. The lowest total born and born alive piglets was shown in October ($p< 0.05$).

## *Discussion*

This study demonstrates the semen production in a commercial isolated boar station that serves for artificial insemination in grand-parents and parents stock in Thailand. The season, temperature and humidity affected some periods of semen production of both purebred (Duroc, Pietrain) and crossbred (LY, PD) boars that were presented as PRRS-free herd and also kept in EVAP. This study conformed to Sonderman and Luebbe (2008) that the temperature and humidity could affect the semen production by showing the lowest trash rate or high number of total sperm of semen production during winter. Moreover, the negative effect of high temperature on semen quality was also observed during hot summer (Kunavongkrit et al., 2005). According to Sonderman and Luebbe (2008), exceeding $29^0$C ($85^0$C) with 85% humidity affect spermatogenesis. In general, the first indication of abnormal sperm production is observed after the onset of high temperature and when motile sperm do not return to a normal ration until 5 weeks due to spermatogenesis (Yang et al. 2010).

Selection of boars, used in tropical climates, based on sperm production is therefore important because Thailand is located in the tropical area. Nowadays, to control the fluctuation of inside temperature EVAP system is adapted for housing; however, the number of hot days can still be recorded over and over. Male animals that have been suffering several hot days critical effect on total number of sperm per ejaculate (Auvigne et al., 2010). According to semen output of Duroc and Pietrain suffering from several hot days were critical to be show as lower quality according to crossbred PD as well than the others in this study. On the other hand, crossbred LY had no significant difference. In wild boar such as Duroc high semen volume and high total number of sperm per ejaculate were found in November and December (Kozdrowski et al., 2004).

The sperm production also differed among the breeds. According to Park and Yi, (2002) Yorkshire boars produced higher semen volume compared to Duroc boars among seasons. This result resembles Wolf and Smital (2009) who revealed that the reduction in volume could be seen significantly different in Duroc when compared among breeds. Generally, Duroc had the lowest semen volume and highest sperm concentration, whereas the sire line of Yorkshire had the highest semen volume and the lowest sperm concentration. Trudeau and Sanford (1990) suggested that the volume of semen can be altered in adult Landrace boar by season which that decreased in semen quality precede summer so that increasing in ambient temperature and the presence or absence of female pigs alters seasonal patterns.

Purebred boars were more sensitive to fluctuation of temperature than crossbred in the volume and total number of sperm per ejaculation, which tended to be lower in purebred during the summer, agreeing with Sonderman and Luebbe (2008). In Thailand, high humidity during rainy (Jun-Oct) might contribute to the seasonal influence on the sperm output. Meanwhile, sperm output varied with season, including high values in autumn and winter and low ones in spring and summer in the Czech Republic (Smital et al., 2004; Smital, 2005; Smital, 2010). Even though there was a report that boars exposed to increasing or decreasing photoperiod between seasons could affect sperm concentration due to testicular function (Sancho et al, 2004), in this study there was slightly decrease concentration in winter. It is assumed that Thailand, which is located in the tropical zone, has no effect of photoperiod on boars kept in EVAP system.

Suriyasomboon et al. (2006) revealed that the pattern of seasonal effect on reproductive performance in tropical area differed from that found in northern Europe and USA due to the differences in seasons. Normally, there are three seasons in tropical area (summer, rainy and winter) while there are four seasons in northern Europe and USA (winter season: Dec to Feb, spring: Mar to May, summer: Jun to Aug and autumn: Sept to Nov). Since swine gestation period spends approximately four months, farrowing at that time of season come from the insemination using semen during the previous season as sequence (summer, rainy and winter). From this study, these corresponded to insemination occurred in middle to late winter (Jan, Feb), the increase of total piglets born was found during summer. On the other hand, the insemination occurring during summer to early rainy season shows the lower production all period of rainy. The good or bad fertility data in tropical area could be caused by semen quality, however, one must consider that poor fertility data might be affected by the factors from female such as ovarian function, stress resulting to abortion or management.

***Conclusion:*** Breed can affect semen production in PRRS free boars living in EVAP while season can affect semen in some period especially during high humidity as rainy. The semen production of crossbred LY boars in Thailand was superior to Duroc and Pietrain boars. To produce good semen production and high fertility, PRRS free status is necessary to perform the good health status.

## *Acknowledgements*

Financial support for this study was provided through the Thailand Research Fund (TRF) in the part of Royal Golden Jubilee (RGJ) industrial linked 2007. The authors would like to thank the Pig Business in Charoen Phokphand Food (CPF) for the available data and the staffs of AI boar stud for excellent data collection.

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