Investigation of the Seasonal Variations of Temperature and Relative Humidity on Fertility Potential of Indoor Boars: Preliminary Data

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Introduction: Numerous environmental factors such as seasonal variations of temperature, daylight length, and relative humidity negatively affect fertility potential of livestock animals. The impacts of such variations on reproductive performance of farm animals are referred to as seasonal infertility, which is characterized with reduced fertility of both females and males. This phenomenon usually occurs during or after hot summer months or exposure to high temperatures, which has been clearly demonstrated with outdoor animals. Although farming in pastures (outdoor) is more affordable to producers, this housing system, however, provides minimal control of the environment, which frequently leads to seasonal infertility of the herds. Unfortunately, the occurrence of seasonal changes remains unpredictable, which situation has forced producers to adopt different indoor housing (farming) systems to mitigate the impacts of seasonal variations in livestock production. Especially in commercial pig operations, sophisticated and expensive temperature-regulated barns are common in boar studs, and yet the optimal protection of boar fertility in such housing system may still not be reached. Indeed, numerous indoor boars are still subjected to high variations in their semen production outputs, and therefore, their consistent field fertility throughout the year. ***Our hypothesis*** is that despite their housing in environment-controlled buildings, indoor boars may still be experiencing the effects of seasonal variations, leading to yearly variations of their field fertility after artificial inseminations. Thus, ***the objective*** of the study was to monitor the seasonal variations of temperature and relative humidity of a commercial seed stud barn and investigate the potential impacts of these variations on semen production and quality of housed boars.

Materials and Methods: Data were collected from Prestage Farms, a commercial hog operation that is located 32.7 miles away (West Point, MS) from our laboratory, at Mississippi State University. In experiment 1, we used temperature and relative humidity (RH) sensor devices (HOBO data loggers; Onset, Bourne, MA) that were placed both outside and inside the barn, in the vicinity but out of reach of boars. Both HOBO data loggers were calibrated to log temperature and RH at 30 minutes intervals, from November 2013 to February 2014. Recorded data were uploaded every week (after semen collection) to a computer for data processing with the HOBOware Professional 3.4.1 software. External temperature profiles were compared to those obtained from the National Weather Service (www.weather.gov) or The Weather Channel (www.weather.com) to validate our recording system. In experiment 2, we used a Digital Infra-red Thermo-Imaging (DITI) camera (FLIR ThermoCAM S60; FLIR Systems, Inc., Boston, MA) to measure the scrotal surface temperature gradient, as an effective tool to evaluate with noncontact means, and its impact of seasonal variations on testicular functions. To this end, a total of ten proven fertile boars within the barn were randomly selected for the study. Images were taken before, during and after each semen collection and were uploaded to a computer and processed with the ThermaCAM Researcher Professional v2.7 software. In experiment 3, semen was harvested twice a month from all boars (n=10). Semen production outputs (volume, concentrations and total sperm number) were evaluated and sperm motility characteristics were assessed within our laboratory with the Computer-Assisted Sperm Analyzer (CASA). Subsets of each boar's semen were frozen in 0.5 ml plastic straws using manual method, while others were centrifuged through a percoll gradient to purify spermatozoa. Both frozen straws and pellets of purified spermatozoa were stored at -196^{o}C (liquid nitrogen) and -20^{o}C, respectively, for further molecular analyses. All data were statistically analyzed using the General Linear Model (Multivariate or Univariate) of the IBM SPSS statistic package. Calendar months (November, December, January, and February), boars, and semen collections were considered as fixed factors to analyze the temperature and relative humidity variations throughout the months, as well as their impacts on scrotal (testicular) temperatures, semen production outputs of boars and sperm motility characteristics. The threshold of significance was fixed at P <0.05.

Results: Data revealed that external temperature profiles recorded in the current study (local) entirely mirrored those obtained from the National Weather Service reports (Regional). The similarity of these observations confirmed the accuracy of our recording system (P > 0.05). Results of experiments 1 and 2 are summarized in Table 1. This table shows that throughout the experimental period, the average temperature and relative humidity data recorded inside the barn were 20.2^{o}C ± 0.4^{o}C and 49% ± 1.2%, respectively, while the outside were 7.4^{o}C ± 0.4^{o}C and 70% ± 1.2%, respectively. Differences between inside and outside values (temperatures and relative humidity) were highly significant, with a P value less than 10^{-4}. These averages hide significant differences that were observed between months (P < 0.05). Indeed, the outside measurement of environmental changes indicated significant fluctuations of both temperatures and relative humidity throughout days and months (P<0.05), with both January (5.5^{o}C ± 0.7^{o}C) and February (4.4^{o}C ± 0.8^{o}C) being the coldest months. The average data of both months were not statistically different (P > 0.05). Inside the barn's data were interestingly, as recorded temperatures were steady throughout the entire experimental period (20.2^{o}C ± 0.4^{o}C; P > 0.05), despite external temperature variations. The relative humidity within the barn highly fluctuated and followed the pattern of the outside values, while remained consistently lower (P > 0.05). We did not observed any statistical differences on scrotal temperature variations across months (P = 0.411) in experiment 2. However, the ranges of these variations indicated the existence of boars with lowest testicular temperatures during the coldest months (Table 1). In experiment 3, we found that environmental variations of temperature and relative humidity throughout the months did not affect the semen production outputs, although the total sperm number per ejaculate was numerically decreased during the cold months (Table 2; P > 0.05). Although semen volumes, sperm concentrations, and total sperm number were comparable between months (P = 0.68, 0.22, and 0.631, respectively), there was a significant interaction between individual boars and months. This interaction indicates that environmental variations differentially affect the currently used boars in terms of sperm concentrations (P = 0.003), leading to a tendency of decreased total sperm production per ejaculate across months (P = 0.09). In experiment 3, the proportions of motile sperm and velocity characteristics were significantly affected (Table 3). Although the total sperm motility was comparable between months, the lowest proportions of spermatozoa moving faster and progressively (or straightforward) were found during the cold month of January. Complex effects of temperature and relative humidity variations were also observed on commonly used velocity parameters (average path – VAP -, straightline – VSL -, and Curvilinear – VCL- velocities and straightness and linearity) of spermatozoa that may influence their fertilization potential.

Table 1. Evaluation of environmental and boar scrotal temperature variations across months

Months	**Climatic Parameters (at the boar stud)**			**Scrotal temperature**
		Inside	**Outside**	[Minimal to Maximal]
November	Temperature (^{o}C)	20.6 ± 0.7	10.8 ± 0.7 ^{a}	30.9 ± 0.2
	RH (%)	55.1 ± 2.4 ^{ac}	72.0 ± 2.4	[30.5 to 31.3]
December	Temperature (^{o}C)	20.4 ± 0.8	9.1 ± 0.7 ^{a}	31.2 ± 0.2
	RH (%)	53.5 ± 2.6 ^{ab}	79.5 ± 2.6	[30.7 to 31.6]
January	Temperature (^{o}C)	19.7 ± 0.7	5.5 ± 0.7 ^{b}	30.8 ± 0.3
	RH (%)	40.1 ± 2.4 ^{b}	56.2 ± 2.4	[30.3 to 31.4]
February	Temperature (^{o}C)	20.1 ± 0.7	4.4 ± 0.8 ^{b}	31.3 ± 0.2
	RH (%)	47.5 ± 2.5 ^{c}	74.3 ± 2.6	[30.9 to 31.7]
Overall averages	Temperature (^{o}C)	20.2 ± 0.4	7.4 ±0.4	
	RH (%)	49.0 ± 1.2	70.0 ± 1.2	
Global effects of the location (inside or outside) and months		$< 10^{-4}$	$<10^{-4}$	0.411
Significant interactions between month and location: Temperatures ($P < 10^{-4}$) and Relative Humidity (P = 0.047)				

Values with different letters within the same column indicate significant differences (P<0.05)

In conclusion, our findings indicate that: **1.** The environment-controlled commercial barn accessed in this research project (***i***) is able to maintain a stable inside barn temperature despite the outside changes and (***ii***) does not regulate relative humidity environment, which may not yet have detectable impacts on reproductive performance of boars; **2.** The DITI camera may be an alternative and reliable measurement tool to evaluate the direct impact of seasonal variation of temperatures and relative humidity on boar testes; **3.** Although there were no statistically significant effects of the month (temperature) on sperm concentrations and total sperm number, the reduction on total sperm may have considerable effect on prepared semen doses available for artificial inseminations, leading to economic loss of the boar stud. Most importantly, there were subsets of boars maintaining high semen production or variable production regardless of the month (or temperature variations). Because the motility characteristics of spermatozoa appeared not strongly related to the monthly temperature variations, the development of novel molecular-based methods to identify the aforementioned subsets of boars (thermo sensitive and thermo-neutral or -resistant) may constitute an important task to achieve and which will certainly lead to major impact in the swine production, as farmers can only focus their production towards the exclusive farming of thermo-resistant boars. We intend to continue our effort toward this objective by investigating each boar through the use of frozen straws and sperm pellets saved in this project.

Table 2. Impact of monthly environmental variations on boar semen production

Months (Outside temperature)	**Semen characteristics**		
	Volume (ml)	**Concentrations (x 10^6/ml)**	**Total sperm (x10^9)**
November (10.8 °C)	265 ± 10	391 ± 25	102
December (9.1 °C)	264 ± 10	388 ± 25	101
January (5.5 °C)	244 ± 10	396 ± 26	96
February (4.4 °C)	360 ± 10	360 ± 25	86
Global effects of the months:	0.68	0.622	0.631
Month*Boar interactions	0.295	0.003	0.089

Table 3. Impacts of monthly environmental variations on velocity parameters of boar spermatozoa

Months (Temperature in °C)	**Velocity parameters**				
	VAP (µm/s)	**VSL (µm/s)**	**VCL (µm/s)**	**Straightness (%)**	**Linearity (%)**
November (10.8 °C)	65 ±1.6 ^{c}	40 ± 1 ^{b}	138 ±3 ^{c}	61 ± 0.8 ^{ac}	31 ±0.5 ^{a}
December (9.1 °C)	70 ±0.9 ^{a}	43 ±0.6 ^{a}	147 ±1.7 ^{a}	61 ±0.5 ^{ac}	31 ±0.3 ^{a}
January (5.5 °C)	68 ±0.9 ^{ac}	40 ±0.6 ^{b}	143 ±1.8 ^{ac}	59 ±0.5 ^{c}	30 ±0.3 ^{a}
February (4.4 °C)	75 ±0.9 ^{b}	40 ±0.6 ^{b}	161 ±1.8 ^{b}	53 ±0.5 ^{b}	26 ±0.3 ^{b}
Month effect: $P < 10^{-4}$			Boar effect: $P < 10^{-4}$		

In all Tables, values with different letters within the same column indicate significant differences (P<0.05)

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