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Effect of Season on Hampshire Crossbreed Boar Semen Quality and Antioxidant Status under Hot Humid Sub-Tropical Climate

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ABSTRACT

The present investigation aimed to study the season's effecton semen quality,and antioxidant status of Hampshire crossbred boar in sub-tropical climatic condition. For the present experiment, same six Hampshire crossbreed boars were used in summer and winter season. Sperm quality parameters (SQPs) were assessed microscopically. Computer-assisted semen analysis (CASA) was used to assess sperm kinematics. Antioxidants biomarkers (glutathione peroxidase; GPx, catalase; CAT, total antioxidant capacity; TAC) and lipid peroxidation (malondialdehyde; MDA) were measured in boar's seminal plasma. Summer season had significant ($p < 0.01$) negative effect on reaction time and false mounts. Semen volume and sperm concentration was recorded to be significantly ($p < 0.01$) higher in winter season. Sperm total motility, progressive motility, viability, acrosomal integrity and HOST reactive sperm were recorded to be significantly ($p < 0.01$) higher in winter season. Season had a significant ($p < 0.05$) effect on the sperm velocity attributes.Seminal plasma antioxidant biomarkers (TAC, MDA, CAT and GPx) were significantly ($p < 0.01$) affected by summer season. In conclusion, the Hampshire crossbreed boar's semen quality and antioxidant biomarkers were negatively affected in summer season. There is need of intervention in terms of genetic, nutrition and management to optimize the boar fertility during summer months in sub-tropics.

***Keywords*:** Boar, Heat Stress, Semen Quality, Antioxidant, Biomarkers, Sub-Tropical.

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INTRODUCTION

Pigs are sensitive to heat stress because of their high metabolism, poorly developed thermoregulatory system, lack of functional sweat glands, and have large deposit of subcutaneous fat which impairs the loss of heat by sweating (Vermeer and Aarnink, 2023). With climate change and increase in summer temperature, animal welfare, health

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and production is compromised (Ross *et al.,* 2015). The micro-climate in the animal farm is more affected during high ambient temperature (Mikovits *et al.,* 2019). In tropics and sub-tropics, the temperature and humidity during summer months are quite high as compared to temperate climate. This leads to high temperature humidity index (THI) which is a significant heat stressor particularly to pigs. Moreover, reliance on temperate pig breeds for breeding and commercial purpose in tropical and sub-tropical climate may leads to sub-optimal performances of these breeds because of high THI (above 72).

Heat stress in pigs causes changes in physiological and behavioural ways to maintain homeothermia and it adversely affects the reproductive physiology and fertility of the boar (Cabezona *et al.,* 2016; Shahat *et al.,* 2020; Singh *et al.,* 2021; Singh *et al.,* 2023). It has previously been reported that alteration in photoperiod and temperature has a significant effect on boar reproductive efficiency (Cheon *et al.,* 2002; Pinart and Puigmulé, 2013; Fraser *et al.,* 2016; Singh *et al.,*2022). The current breeds of the pigs have inherited reproductive seasonality from its ancestor, the European wild boar (*Sus scrofa ferus*) (Kozdrowski and Dubiel, 2004).Environmental induced heat stress reduces the boar semen quality and fertility in hot humid climate (Singh *et al.,* 2021; Singh *et al.,* 2022a).High temperature-humidity index (THI) leads to alteration in boars' physiology and subsequently leads to decline in boar sperm quality and fertility (Knecht e*t al.,* 2013; Pinart *et al.,* 2013; Fraser *et al.,*2016; Gallardo *et al.,* 2019; Martín-Hidalgo *et al.,* 2020). It has been proposed that spermatogenesis and germ cells are more vulnerable to heat stress-induced cellular damage (Kim *et al.,* 2013). Heat stress affects the hypothalamus pituitary gonadal axis and leads to decreased testosterone concentration in blood and in testis which is critical for spermatogenesis and maintaining testicular integrity (Mete *et al.,* 2012). Shahat *et al.* (2020) stated that heat stress accentuates oxidative stress in general, causes germ cell apoptosis, and induces sperm DNA damage. Furthermore, boar sperm have low cholesterol to phospholipids ratio and this make the sperm membrane susceptible to oxidative damage during liquid storage (Baishya *et al.*, 2018; Singh *et al.,* 2021). Also, antioxidant capacity of boar spermatozoa declined during liquid storage in general and in adverse weather particularly. Consequently, boar sperm are more prone to membrane lipid peroxidation and apoptosis during liquid storage (Singh *et al.,* 2021; Singh *et al.,* 2023). Hence, the present study was aimed to assess the effect of different seasons (summer and winter) on semen quality parameters (SQPs) and antioxidant bio-markers in seminal plasma of boar in sub-tropical climate.

MATERIALS AND METHODS

The study was conducted at Pig Research Farm, ICAR Nagaland Centre, Medziphema, Nagaland, India. The location of research farm is 25°45' N latitude,93°50' E longitude and an altitude of 281 meters AMSL. The study was carried out in the summer (June to July) and winter seasons (December to January). In general, the region has sub-tropical climate with 1500 mm to 2000 mm annual average rainfall. The maximum and minimum temperature was 33.6 °C and 24.9 °C in the summer whereas it was 24.6 °C and 9.0 °C in the winter. The maximum and minimum relative humidity was 92% and 71% in summer and 96% and 62% in winter.

For the present study, six Hampshire crossbred boars (Hampshire × Gunghroo) aged 18 to 28 months were used. Boars were housed individually in well-ventilated concrete pens (9 sq. mtr area) with the provision of an open space. Animals were maintained under uniform managemental condition. Boars were fed a commercial breeder feed with 3.0 kg feed/day. The boars had ad *libitum* access to drinking water. Semen was collected by the gloved-hand technique once in a week. In total 30 ejaculates were collected from six boars (five ejaculates from each boar) in summer as well as in winter (a total of 60 ejaculates in both seasons). Ejaculates were transported to the laboratory in thermos flask immediately after collection. In laboratory, semen samples were examined at the fresh stage (details given in next paragraph) and then extended in Primxcell (IMV, France) medium maintained at 37 °C. The semen was so diluted that each 80 ml semen pouch contains 3 billion total motile spermatozoa. Semen pouched were sealed and kept at 17 °C in a BOD incubator for 72 h. Semen samples were then examined after 72 h of storage, for sperm quality parameters.

Reaction time and false mount were recorded as described earlier (Singh *et al.,* 2021). Semen volume (mL) was recorded using a graduated glass cylinder. Sperm concentration (millions per mL) was determined using haemocytometer methods (Liu *et al.,* 2015). The sperm viability was determined by adopting a differential staining technique using Eosin-Nigrosin stain. Sperm abnormalities like abnormal heads, abnormal tails, abnormal midpieces, detached heads, coiled tails, and presence of proximal cytoplasmic droplets were observed (Shipley, 1999). The Giemsa stain method was used to assess sperm acrosome integrity. Spermatozoa plasma membrane integrity was assessed by hypo-osmotic swelling test (Singh *et al.,*2021). Computer-assisted semen analysis (Hamilton

Thorne Sperm Analyzer (HTM-IVOS, version IVOS 11, Hamilton Thorne Research, USA) was used to assess sperm kinematic parameters *viz.* average path velocity (VAP), straight-line velocity (VSL), curve linear velocity (VCL), the amplitude of lateral head displacement (ALH), beat cross frequency (BCF), straightness (STR), and linearity (LIN).

Seminal plasma antioxidants biomarkers (GPx, TAC, and CAT) and lipid peroxidation (MDA) were estimated in summer and winter using Cayman's kit. For this, a portion of the semen ejaculate was immediately centrifuged once at 1000 X g/min for 15 min. Seminal plasma was collected and stored in 2.0 mL Eppendorf (EP) tubes at -20 °C for further analysis. Thermo Scientific Multiskan GO Microplate Spectrophotometer, USA was used for measuring absorbance of samples.

The data were checked for normal distribution by Shapiro-Wilk test. Statistical analysis was done using IBM Statistical Package for the Social Sciences (SPSS) v27. For comparing the means of two group, the data were analyzed using independent sample t-test. Results are presented as mean ± SEM and differences were considered significant at $p < 0.05$.

RESULTS AND DISCUSSION

Summer season had significant ($p < 0.01$) negative effect on reaction time and false mounts (Table 1). Semen volume and sperm concentration was significantly ($p < 0.01$) higher in winter season. Similarly, semen quality parameters (SQPs) were significantly ($p < 0.01$) higher in winter season. There was a significant ($p < 0.05$) effect of season on the sperm kinematics *viz.* VAP, VSL, VCL, ALH and BCF (Table 2), however, STR and LIN did not differ significantly ($p > 0.05$) between the two seasons.

Table 1: Effect of season on boar semen quality at the fresh stage (mean±SE).

Parameters	Summer	Winter	P-value
Reaction time (min)	4.30 ± 0.16^{A}	2.71 ± 0.18^{B}	<0.001
False mount (numbers)	2.18 ± 0.12^{A}	1.53 ± 0.10^{A}	<0.001
Semen volume (mL)	155.80 ± 2.40^{B}	206.06 ± 3.08^{A}	<0.001
Sperm concentration (million per mL)	122.70 ± 1.65^{B}	147.66 ± 1.74^{A}	<0.001
Total sperm per ejaculate (billions)	19.13± 0.41	30.45± 0.62	<0.001
Sperm total motility (%)	74.56 ± 0.75^{B}	86.33 ± 0.86^{A}	<0.001
Sperm progressive motility (%)	31.63 ± 0.44^{B}	35.96 ± 0.39^{A}	<0.001
Viability (%)	80.26 ± 0.56^{B}	90.40 ± 0.67^{A}	<0.001
Abnormality (%)	12.86 ± 0.23^{A}	8.66 ± 0.27^{B}	<0.001
Acrosomal integrity (%)	77.93 ± 0.58^{B}	87.86 ± 0.68^{A}	<0.001
Hypo-osmotic swelling test (%)	77.96 ± 0.54^{B}	86.63 ± 0.65^{A}	<0.001

^{AB}Values with different superscripts in a row differ significantly ($p < 0.05$). n=30 in each season.

Table 2: Effect of season on boar sperm kinematics at the fresh stage (mean±SE).

Parameters	Summer	Winter	P-value
VAP (µm/sec)	56.80 ± 0.27B	61.73 ± 0.58A	<0.01
VSL (µm/sec)	45.50 ± 0.24B	49.00 ± 0.39A	<0.01
VCL (µm/sec)	118.86 ±0.88B	124.56 ±3.02A	0.012
ALH (µm)	5.22 ± 0.04B	5.80 ± 0.03A	<0.01
BCF (Hz)	24.12 ± 1.05B	27.66 ± 0.34A	<0.01
STR (%)	0.80 ± 0.00A	0.79 ± 0.01A	0.20
LIN (%)	0.38 ± 0.00A	0.42 ± 0.03A	0.33

^{AB}Values with different superscripts in a row differ significantly ($p < 0.05$). n=30 in each season.

Semen quality parameters were significantly ($p < 0.01$) higher after liquid storage in winter as compared to summer season (Table 3). There was a significant ($p < 0.05$) effect of season on sperm kinematic parameters *viz.* VAP, VCL, ALH, BCF, STR and LIN (Table 4).

Table 3: Effect of season on boar sperm quality after liquid storage (mean±SE).

Parameters	Summer	Winter	
Sperm total motility (%)	65.00 ± 0.47^{B}	74.63 ± 0.61^{A}	<0.01
Sperm progressive motility (%)	25.10 ± 0.26^{B}	31.90 ± 0.42^{A}	<0.01
Viability (%)	72.90 ± 0.42^{B}	80.70 ± 0.47^{A}	<0.01
Abnormality (%)	17.36 ± 0.39^{A}	12.26 ± 0.33^{B}	<0.01
Acrosomal integrity (%)	69.43 ± 0.43^{B}	77.60 ± 0.40^{A}	<0.01
Hypo-osmotic swelling test (%)	68.66 ± 0.41^{B}	75.76 ± 0.32^{A}	<0.01

^{AB}Values with different superscripts in a row differ significantly ($p < 0.05$). n=30 in each season.

Table 4: Effect of season on boar sperm kinematics after 72 hrs of storage at 17 °C (mean±SE).

Parameters	Summer	Winter	P-value
VAP (µm/sec)	54.46 ± 0.42^{B}	56.63 ± 0.75^{A}	<0.01
VSL (µm/sec)	43.20 ± 0.36^{A}	43.50 ± 0.31^{A}	0.58
VCL (µm/sec)	110.36 ± 1.32^{B}	122.70 ± 0.75^{A}	<0.01
ALH (µm)	4.88 ± 0.03^{B}	$5.16 \pm 0.0.03^{A}$	<0.01
BCF (Hz)	21.49 ± 0.23^{B}	24.76 ± 0.29^{A}	<0.01
STR	0.79 ± 0.00^{B}	0.77 ± 0.01^{A}	0.01
LIN	0.39 ± 0.00^{A}	0.35 ± 0.00^{B}	<0.01

^{AB}Values with different superscripts in a row differ significantly ($p < 0.05$). n=30 in each season.

Previous studies have documented the season dependent changes in boar semen quality (Pinart and Puigmulé, 2013; Wilczyńska *et al.,* 2013; Fraser *et al.,* 2016). Chen *et al.* (2017) reported similar findings in boars under high ambient temperature in China. Ren *et al.* (2009) and Chen *et al.* (2017) reported that heat stress because of high ambient temperature causes decline in serum testosterone and estradiol concentration and thereby leads to reduced boar's libido. Similarly, Kunavongkrita *et al.* (2005) reviewed effect of heat stress in boar in Thailand and reported that during heat stress, crude protein intake is reduced along with increase in scrotal temperature which may leads to poor libido. Wysoki´nska *et al.* (2023) reported that boar semen in summer season was characterized by low volume and low sperm concentration than ejaculates collected in winter season. In winter, high semen volume and higher sperm counts Ejaculates were because of favourable ambient temperature to spermatogenesis (Knecht *et al.,* 2014; Wysoki´nska *et al.,* 2023).

The better sperm quality and sperm kinetic parameters during winter season allow for optimal utilization of high genetic value boars whereas summer season restrict their efficient utilization in sub-tropical climate. Fraser *et al.* (2016) reported better sperm quality in boars during autumn-winter period. Similarly, previous studied documented that sperm motility, viability, morphology and acrosome integrity were significantly decreased in the summer or during the long photo period (Zasiadczyk *et al.,* 2015; Wysoki´nska *et al.,* 2023). Boar fertility decreased in summer because of heat stress (Zasiadczyk *et al.,* 2015). Parrish *et al.* (2017) reported that boar subjected to prolonged exposure to high temperatures have more consequences of heat stress on their semen quality. Wysoki´nska *et al.* (2023) reported that liquid storage of boar sperm at 17°C resulted into morphological changes in the sperm in a season dependent manner. It is known that boar semen quality deteriorates during liquid storage, however, it deteriorates more rapidly during summer months. Heat stress negatively affects every stage of spermatogenesis process and it may damage sperm DNA (Parrish, 2019; Flowers, 2022).

Seminal plasma TAC was significantly ($p < 0.01$) low in winter season (Table 5). Seminal plasma MDA concentration was significantly ($p < 0.01$) increased during summer season. There was a significant ($p < 0.051$) effect of season on seminal plasma, GPx, and CAT. This finding partly explains the poor quality of boar semen during summer season. Increased ROS production during summer season affects the sperm quality and fertility. ROS affects spermatogenesis, sperm maturation, mitochondria function, sperm membrane and sperm DNA. In agreement with our findings, Chen *et al.* (2017) reported similar finding in boar semen and dietary L-arginine supplementation improved semen total antioxidant capacity, glutathione peroxidase and catalase activities. The high heat stress might lead to supraphysiologic reactive oxygen species (ROS) production, which may compromise sperm structural integrity and functional competence (Esfandiari *et al.,* 2002; Lavranos *et al.,* 2012). It has been previously reported that oxidative stress damages the sperm DNA integrity (Agarwal *et al.,* 2016). Pena *et al.* (2019) reported that tropical summer induces DNA fragmentation in boar spermatozoa. Testicular temperatures of boars are maintained 4°C to 6°C lower than core body temperature for optimal spermatogenesis (Setchell, 2006). However, high ambient temperature may raise testicular temperature which has a detrimental effect on spermatogenesis and the resultant spermatozoa.

Table 5: Effect of season on antioxidant biomarkers in boars' seminal plasma (mean±SE).

Parameters	Summer	Winter	P-value
TAC (mmol/L)	0.37 ± 0.00^{B}	0.41 ± 0.00^{A}	<0.01
MDA (nmol/mL)	1.89 ± 0.00^{A}	1.73 ± 0.00^{B}	<0.01
GPx (nmol/min/mL)	80.03 ± 0.85^{B}	104.76 ± 0.92^{A}	<0.01
CAT (nmol/min/mL)	4.83 ± 0.07^{B}	6.79 ± 0.04^{A}	<0.01

^{AB}Values with different superscripts in a row differ significantly ($p < 0.05$). n=30 in each season.

CONCLUSION

In conclusion, the findings of the present study revealed important insights into the heat stress induced reproductive seasonality of Hampshire crossbreed boar in Indian condition. Summer season significantly compromised the boar's libido, semen volume, sperm concentration, sperm quality, sperm kinetics and antioxidant biomarkers

in sub-tropical climates. Total sperm per ejaculate were higher in winter season which allows more efficient utilization of boars in AI programmes. As the environment temperature and humidity is very high in sub-tropical climate, therefore, necessary interventions in the form of genetics, management and nutrition are needed for optimal utilization of breeding boars.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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