Effect of Season Influencing Semen Characteristics, Frozen-Thawed Sperm Viability and Testosterone Concentration in Duroc Boars

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ABSTRACT : This study was carried out to investigate the effects of season influencing semen characteristics, frozen-thawed sperm viability and testosterone concentration in Duroc boars. There were no significant differences in the semen volume and sperm concentration of Duroc boars among spring, summer, autumn and winter. However, the pH of sperm-rich and sperm-poor fractions in autumn and winter season was higher than in spring and summer season in Duroc boars. Sperm motility and normal acrosome of raw semen in Duroc boars did not differ significantly among spring, summer, autumn and winter. However, motility and normal acrosome of frozen-thawed sperm were higher in spring season than in summer, autumn and winter. Serum testosterone concentrations in Duroc were higher in spring than summer, autumn and winter. In conclusion, when serum testosterone concentrations were higher in seasons, frozen-thawed sperm viability in Duroc boars were higher. ***(Asian-Aust. J. Anim. Sci. 2002. Vol 15, No. 4 : 500-503)***

Key Words : Season, Sperm Viability, Testosterone, Duroc, Boar

INTRODUCTION

Semen production of boars is influenced by many factors, such as breed, age, nutrition, environmental effects, health status and frequency of use, which result in great variations of semen characteristics (Hughes and Varley, 1980). Since the application of artificial insemination in animals, there has been a growing interest and necessity for more knowledge concerning variation of semen characteristics. Boars consistently producing high-quality ejaculates are crucial.

Seasonal effects on both male and female pig reproduction have been reported (Claus and Weiler, 1985a). In boars, seasonal changes were influenced by photoperiod (Claus and Weiler, 1985a) and temperature (Mazzari et al., 1968). The latter effect was primarily a deleterious effect of high temperature causing germ-cell destruction (Mazzari et al., 1968) and resulting in a temporary decrease in sperm production and fertility (Roller et al., 1973; Wettemann and Bazer, 1985). Borg et al. (1993) reported that characteristics of sperm morphology did not differ among breeds of boars. Although season had significant effects on percent live sperm (highest in Autumn) and percent sperm with normal acrosomes (lowest in winter), the differences among seasonal means for these sperm characteristics were too small to be considered biologically important.

Leman and Rodeffer (1976) reported that puberty occurred at between 5 and 8 months, and the number of sperm and the volume of ejaculate increase until the boar reaches 18 months. The ejaculate at this time was between 20 and 80×10^9 sperm in 200-400 ml of semen. This level of sperm production was maintained until a gradual decline started after year 5 of the boar's life. Diehl et al. (1979) reported that the number of sperm and the volume of ejaculate was $30\text{-}60\times10^9$ sperm and 150-200 ml, respectively. Von Rohloff (1973) reported that there were no differences of semen volume between 1 to 4 years of age in boars. There were significant between breed differences in sperm production and generally the larger breeds such as the Yorkshire and Large White tended to produce a greater volume of semen per ejaculate and greater numbers of sperm cells over a period, although it is not clear how mature size affects sperm concentration (Hughes and Varely, 1980).

Severe spermatogenic damage occurred when testicular temperature reached 40.5°C (Mazzari et al., 1968) and as body temperature was closely related to testicular temperature, any disease associated with high body temperature might damage testicular tissue directly and therefore induce infertility. Cold temperature conditions on the other hand do not generally adversely affected semen quality or fertility (Swiestra, 1970).

Steroid determination on the testis (Elsaesser et al., 1972; Booth, 1975) and peripheral blood (Elsaesser et al., 1976; Colenbrander et al., 1978; Tan and Raeside, 1980) had shown that relatively high levels of androgen were present during the postnatal period, decreasing during early puberty before increasing again between late puberty and maturity. In the peripheral blood of adult boar, testosterone was usually>2 ng/ml.

Sperm production of boars may fluctuate up as much as 25-30% through out the year, forcing AI centers to keep additional boars to compensate for these fluctuations

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(Colenbrander and Kemp. 1990). Therefore, better knowledge of factors influencing both quantity and quality of semen may help to improve the efficiency of AI organizations. So. this study was carried out to investigate the effects of season influencing semen characteristics. frozen-thawed sperm viability. and serum testosterone concentration in Duroc boars.

MATERIALS AND METHODS

Semen collection

Semen was collected from January 2000 to December 2000 from adult Duroc boars 15-22 month of age with bodyweight ranging from 150 kg to 200 kg. Boars were housed at Livestock Experiment Station of Chungnam Livestock Sanitation Research Institute in Boryong and were used in an AI program. Semen was collected from each of 5 Duroc boars one time per week. The filtered sperm-rich fraction was collected by the gloved-hand technique into a 250 ml insulated vacuum bottle.

Climatological information for the Livestock Experiment Station during the experimental year is summarized in table 1.

Determination of semen volume, pH and sperm concentration

Semen volumes were determined with a graduated cylinder. pH of the sperm-rich and sperm-poor fractions was determined by a pH meter (WTW GmbH. Germany). Sperm concentrations were estimated by a hemocytometer. The sperm-rich fractions of ejaculates with greater than 80% motile sperm and NAR acrosome were used for frozen semen processing.

Frozen semen processing

The sperm-rich fraction (30 to 60 ml) of ejaculate was collected into an insulated vacuum bottle. Semen was slowly cooled to room temperature (20 to 23°C) by 2 h after collection. Semen was transferred into 15 ml tubes. centrifuged at room temperature or 10 min at 800 g. and the supernatant solution was poured off. One volume of concentrated sperm was resuspended with 1 volume of lactose egg-yolk diluent (the first diluent to provide 1.0×10^9 sperm/ml) at room temperature. Semen was cooled in refrigerator to 5°C over a 2 h period and 1 volume of a LEY+6% glycerol diluent (the second diluent) was added to one volume of cooled semen. Straws (Minitube Gmbh. Landshut. Germany) were immediately filled with 5 ml of semen and steel or glass balls were used to seal the ends of the straws. The air bubble was adjusted to the center of each straw and the straws were horizontally placed on an aluminum rack and set into a liquid nitrogen tank containing liquid nitrogen (LN). The straws were situated 5 cm above the LN. and kept at that level for 20 min before the straws were transferred into LN storage.

Sperm evaluation

Immediately after each straw was thawed in 52°C water bath for 45 sec. 5 ml of their contents were added to 30 ml of BTS (Beltsville thawing solution) at 22 to 24°C. One ml aliquot of the diluted semen was then added to 1 ml of 1% glutaraldehyde in BTS for the 0 h acrosome morphology evaluation and 29 ml were incubated at 37°C. After 30 min incubation, 10 ul aliquots were transferred onto glass slides and 18×18 mm cover-slips were applied. The percentage of motile sperm was estimated at 37°C by light microscope at 250×.

The acrosome morphology of 100 sperm per sample at 0 min after thawing was evaluated by phase contrast microscopy at 1000×. Acrosome were differentially categorized into four morphological classes normal apical ridge (NAR), damaged apical ridge (DAR), missing apical ridge (MAR) and loose acrosomal cap (LAC) as described by Pursel et al. (1972). All samples were coded at thawing and the identity of the treatment was unknown during evaluation.

Blood collection and analysis of serum testosterone

Blood samples were collected every two weeks from January 2000 to December 2000 from adult Duroc boars 15-22 months of age. Blood samples were obtained at 10 a.m. by inserting a 21-gauge, hypodermic needle attached to 10 ml syringe through surface veins in the ear. Blood samples were allowed to clot at 4°C and serum was obtained by centrifuged for 15 min at 2,000 rpm. The serum was then stored at -20°C.

The concentration of testosterone was determined by Competitive Enzyme Immunoassay. Aliquots of 50 μl porcine serum were collected into a clean glass test tube.

Table 1. Climatological data for the experimental year at Boryong of Chungnam in Korea[a]

Season	Air temperature (°C)			Relative humidity (%)		
	Mean	Minimum	Maximum	Mean	Minimum	Maximum
Spring (March-May)	10.2	5.1	15.7	60.1	19.5	88.0
Summer (June-August)	24.5	21.2	28.7	75.8	57.3	94.1
Autumn (September-November)	14.8	10.5	20.0	66.8	40.6	83.1
Winter (December-February)	0.5	-3.7	5.2	66.6	38.8	89.1

[a] Entries are means followed by ranges.

The aliquots were added 0.5 ul of diethyl ether and mixed thoroughly with a vortexer. To separate the layers, the ether was removed by freezing the aqueous layer in an ethanol/dry ice bath and decanted. The ether was placed into a clean test tube. The ether was evaporated by heating to 30°C with a gentle stream of dry nitrogen. The extract was dissolved in 50 ul of Enzyme Immunoassay (EIA) buffer.

The EIA kit (Cayman Chemical, MI, USA) was rinsed three times with washing buffer and added 50 ul of testosterone standard and sample per well, respectively.

Testosterone acetylcholinesterase tracer and testosterone antiserum of 50 ul, respectively, were incubated on plate shaker for one hour at room temperature. The wells were emptied and rinsed five times with washing buffer. Ellman's reagent of 200 ul per well was added and developed the plate in one hour and read the plate at 405 nm by Microplate Reader (Molecular Device, USA).

Statistical analysis

Analysis of variance (ANOVA) were carried out using the SAS package (SAS, 1988) in a completely randomized design. Duncans multiple range test was used to compare mean values of individual treatments, when the F-value was significant ($p<0.05$).

RESULTS

Seasonal variations on semen characteristics of Duroc boars collected throughout the year were presented in table 2. There were no significant differences in the semen volume and sperm concentration of Duroc boars among spring, summer, autumn and winter. However, the pH of sperm-rich and sperm-poor fractions in autumn and winter season was higher than in spring and summer season in Duroc boars.

Comparison of motility and normal acrosome of fresh and frozen-thawed sperm during season in Duroc boars were presented in table 3. Sperm motility and normal acrosome of raw semen in Duroc boars did not differ significantly among spring, summer, autumn and winter. However, motility and normal acrosome of frozen-thawed sperm were higher in spring season than in summer, autumn and winter.

Seasonal serum testosterone concentrations in Duroc boars were presented in table 4. Serum testosterone concentrations in Duroc were higher in spring than summer, autumn and winter.

DISCUSSION

In boars, seasonal changes were influenced by photoperiod (Claus and Weiler, 1985a) and temperature (Mazzari et al., 1968). The latter effect was primarily a deleterious effect of high temperature causing germ-cell destruction (Mazzari et al., 1968) and resulting in a temporary decrease in sperm production and fertility (Roller et al., 1973; Wettemann and Bazer, 1985). Borg et al. (1993)

Table 2. Seasonal variations on semen characteristics of Duroc boars

Season[1]	No. of boars	Semen volume (ml)[2]			pH[2]		Sperm concentration[2]	
		Sperm rich	Sperm poor	Total	Sperm rich	Sperm poor	Sperm rich ($\times10^8$/ml)	Sperm poor ($\times10^6$/ml)
Spring	5	98.1	83.2	181.3	7.26^b	7.37^b	3.6	26.8
Summer	5	74.1	69.4	143.5	7.35^b	7.40^b	3.2	31.2
Autumn	5	95.1	77.4	172.2	7.66^a	7.90^a	3.3	28.2
Winter	5	83.3	86.1	169.4	7.65^a	7.75^{ab}	3.3	30.0

[1] Spring (March-May), summer (June-August), autumn (September-November) and winter (December-February).
[2] Means for 3 ejaculates from each of 5 Duroc boars.
[a,b] Means in the same column with different superscripts differ significantly ($p<0.05$).

Table 3. Comparison of motility and normal acrosome of fresh and frozen-thawed sperm during season in Duroc boars

Season[1]	No. of boars	Fresh sperm[2]		Frozen-thawed sperm[2]	
		Motility (%)	Normal acrosome (%)	Motility (%)	Normal acrosome (%)
Spring	5	88.8	93.3	40.9^a	48.0^a
Summer	5	90.0	95.3	19.4^c	22.8^b
Autumn	5	86.3	96.0	35.0^{ab}	26.7^b
Winter	5	88.8	96.5	30.9^b	19.8^b

[1] Spring (March-May), summer (June-August), autumn (September-November) and winter (December-February).
[2] Means for 3 ejaculates from each of 5 Duroc boars.
[a,b,c] Means in the same column with different superscripts differ significantly ($p<0.05$).

Table 4. Seasonal serum testosterone concentrations in Duroc boars

Breed	Serum testosterone concentrations (ng/ml)[1]			
	Spring[2]	Summer	Autumn	Winter
Duroc	3.06[a]	0.73[b]	1.31[b]	1.36[b]

[1] Means for 3 blood collections from each of 5 Duroc boars.
[2] Spring (March-May), summer (June-August), autumn (September-November) and winter (December-February).
[a,b] Means in the same row with different superscripts differ significantly ($p<0.05$).

reported that characteristics of sperm morphology did not differ among breeds of boars. Although season had significant effects on percent live sperm (highest in fall) and percent sperm with normal acrosomes (lowest in winter), the differences among seasonal means for these sperm characteristics were too small to be considered biologically important. Neither season nor breed had any significant effects on percentage of sperm with normal heads or normal tails, or on percentage of proximal droplets.

Our result indicated that season did not affect semen volume and sperm concentration in Duroc boars, but affected pH. Hafez (1978) reported that semen volume, pH and sperm concentration was 150-200 ml, 7.3-7.8 and 2-3×10^8/ml, respectively, which were relevant ranges in this study.

Sperm quality characteristics exhibited clear seasonal changes. The causes of seasonal fluctuations in semen quality are not fully understood, but likely are mediated by hormonal mechanism controlled by photoperiod (Claus et al., 1985b). Clearly an ancient mechanism characteristics for the European wild pig, is still present in modern commercial swine breeds. The European wild pig is a seasonal breeder and does not mate during the summer and fall months (Mauget and Boissin, 1987). For this reason, the lowest reproductive performance of commercial swine is observed in summer (Xue et al., 1994).

In this study, serum testosterone concentrations in Duroc boars were 3.06 ng/ml in spring and 0.73-1.36 ng/ml from summer to winter. Colenbrander et al. (1978) reported that serum testosterone concentration in the peripheral blood of adult boar was 1.77 ng/ml. Park and Lee (1984) reported that serum testosterone concentration was 1.15 ng/ml at 50 kg body weight and remained fairly constant thereafter. However, the seasonal changes of serum testosterone concentration have not been reported with frozen-thawed sperm quality in adult boars.

As a result of this study, we found out that serum testosterone concentrations were higher in seasons, frozen-thawed sperm viability in Duroc boars were higher.

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