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Age and seasonal-dependent variations in the biochemical composition of boar semen

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ABSTRACT

This study investigated the effect of age- and seasonal-related variations in the composition of boar semen over a 3-year period. At the onset of 8 months of age, ejaculates were collected from four boars and allocated into three groups: 8 to 18, 19 to 30, and 31 to 42 months and were divided into two seasonal periods: autumn-winter and spring-summer. Boar variability had a significant effect on most of the analyzed semen parameters. Significantly, higher ejaculate volumes were observed in the boars older than 18 months of age during the autumn-winter period. Sperm concentration was higher in boars less than the age of 18 months of age, whereas the total sperm numbers were significantly higher during the autumn-winter period, regardless of the age group. Seasonal effects in sperm motility were more marked in boars at the age of 19 to 30 months, being significantly higher during the autumn-winter period. The proportions of spermatozoa with intact, normal apical ridge acrosome, and osmotically tolerant acrosomal membranes were markedly higher in boars at the age of 19 to 30 months during the autumn-winter period. Spermatozoa harvested during the spring-summer period were more susceptible to lipid peroxidation, irrespective of the age group. Significantly, higher levels of protein content and concentrations of nonthiol-containing antioxidant components of the seminal plasma (SP) were detected in boars less than 18 months of age during the autumn-winter period. Seasonal effects on the pH and proteinase inhibitory activity in the SP were more marked in boars less than 18 months of age, whereas alkaline phosphatase activity was greater in boars at the age of 19 to 30 months during the autumn-winter period. Substantial amounts of the thiol-containing antioxidants of the SP were detected in boars older than 18 months of age during the spring-summer period. Neither osmolality nor total antioxidant status was affected by differences in the seasonal periods or age groups. The findings of this study indicate that age- and seasonal-related variations affect the reproductive tract functions in the boar, resulting in marked changes in the biochemical composition of the semen.

1. Introduction

The reproductive performance of domestic animals is dependent on many factors, such as the genetic potential, age, breed, season, ambient temperature, nutrition, and management skills [1–4]. Specifically in the boar, these factors have been shown to affect sperm output by approximately 25 to 30% throughout the year [4].

Sexual maturity in the boars occurs at various ages, depending on the breed, and environmental and social cues [2,5–7]. Boars become adolescent at about 9 months old and the semen quality is improved during pubertal development [4,5]. Ejaculates collected from mature boars had better quality than those from the younger than 9 months, and the daily sperm output gradually increased with age,

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being markedly higher in 18-month-old boars compared with younger ones [5,8]. It was reported that the maximum semen quality was obtained from boars at the age of 24–29 months in temperate regions [1]. Age and seasonal variations in hormone levels have been shown to affect the quality of boar semen [5,7,9], and age-dependent increases in testosterone and estrogen levels have been associated with testicular development, puberty, sexual maturity, and sperm production [4,10,11].

Reproductive seasonality, due to variations in photoperiod and temperature, has a marked effect on boar semen quality. It has been reported that the reproductive seasonality in the boar is a trait inherited from its ancestor, the European wild boar (*Sus scrofa ferus*) [12]. There are a variety of studies, which have described the effect of temperature and seasonal variations in the quality of boar semen [3,4,6,10,13–19].

Spermatozoa are suspended in a complex mixture of fluid, the seminal plasma (SP), which comprises various secretions originating from the testes, epididymis, and different accessory sex glands [20–24]. Moreover, boar SP contains a wide variety of biochemical components that are implicated in the sperm-egg fertilization processes [22,24–31]. Accumulating evidence has indicated the concerted action of protein components of boar SP on sperm quality [21,27,29,32,33] and their effects on sperm survival after semen storage at different seasonal periods [10,15,34,35]. Furthermore, a recent study has shown that spermatozoa from ejaculates harvested during the summer are more susceptible to cryo-induced damage, due to marked changes in the SP biochemical composition [36]. It has been suggested that the sperm function is more vulnerable to age- and seasonal-related variations than the secretory activity of the accessory sex glands [5]. More recently, a study from our laboratory has reported on the marked seasonal effects on the sperm quality characteristics and SP proteome in fractionated ejaculates of individual boars [19]. Remarkably, little is known about the effects of age- and seasonal-related changes in the biochemical composition of boar semen. This study investigated the effects of boars of different ages (8–18, 19–30, and 31–42 months) and various seasonal periods (autumn-winter and spring-summer) on sperm quality characteristics and biochemical composition of the SP, including the thiol-containing and nonthiol-containing antioxidants.

2. Materials and methods

2.1. Chemical and media

All chemicals were purchased from Sigma Chemical Company (St. Louis, MO, USA), unless otherwise stated. The bisbenzimide fluorescent probe, Hoechst 33258 (H33258; Calbiochem-Behring Corporation, La Jolla, CA, USA), was purchased from Molecular Probes, (Eugene, OR, USA).

2.2. Animals and semen collections

Four Polish Large White boars, designated as A, B, C, and D, were used in this study. The boars were trained to ejaculate on a dummy sow and ejaculates were collected by one trained technician throughout the entire study. Collections of ejaculates started at the age of 8 months (February), when the boars had reached sexual maturity. The ejaculates were collected from the 4 boars at the age of 8–18 months, 19–30 months, and 31–42 months, using the gloved hand technique. The annual cycle of each age group (8–18, 19–30, and 31–42 months) was divided into two seasonal periods: autumn-winter period (from October to March) and spring-summer (from April to September), as indicated in previous studies [19,28]. Ejaculates were collected from each boar at least 10 to 12 times for a 34-month period, with approximately 7 to 10 days between collections, with the exception of Boar D (Table 1). At the age of 36 months, Boar D had problems with its hind legs and was unable to further participate in the experiment. The boars were housed at the laboratory research facility of the Department of Animal Biochemistry and Biotechnology, Olsztyn, Poland (N: 53° 44′ 35″, E: 20° 27′ 03″). The animals were fed with a commercial porcine ration and were kept in individual pens, under standard environmental conditions. Water was available ad libitum. Animal experiments were carried out in accordance with the guidelines of the Local Ethics Committee.

The volumes of the whole ejaculates, collected into prewarmed graded cylinders, were recorded, and then the gel portion was immediately removed, using double gauze. The gel-free ejaculate volumes were recorded and used to calculate the total sperm numbers.

2.3. Evaluation of sperm quality characteristics

2.3.1. Total sperm motility and concentrations

The percentage of total motile spermatozoa was subjectively assessed by the same technician throughout the study. For the assessments, aliquots (6 μl) of semen samples were placed on prewarmed slide, covered with a glass cover slide (20 × 20 mm), and examined under a light microscope at 200 × magnification (Olympus BX 40, Olympus, Tokyo, Japan) equipped with an attached heated stage (37 °C). Total sperm motility was defined as the percentage of spermatozoa that had any form of motility and was assessed randomly in at least 5 fields.

Table 1
Total number of ejaculates collected from individual boars during at different seasonal periods with various age groups.

Boar	8–18 mo		19–30 mo		31–42 mo	
	Autumn-winter	Spring-summer	Autumn-winter	Spring-summer	Autumn-winter	Spring-summer
A	10	12	12	12	12	12
B	10	12	12	12	10	11
C	10	12	12	12	10	10
D	10	12	12	12	6 (31–36 mo)	6 (31–36 mo)
Total	40	48	48	48	38	39