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Biologic parameters of polar fox (*Alopex lagopus* L.) semen during the breeding season

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Abstract: The aim of this work was to evaluate the biologic properties of polar fox (*Alopex lagopus* L.) semen collected during the reproductive season. In 126 ejaculates manually collected from 18 foxes at 10- to 12-day intervals, semen parameters (such as the sperm concentration, volume, pH, sperm morphology, and activity of acid and alkaline phosphatase) were determined. The seminal plasma acid and alkaline phosphatase activity correlated positively with the sperm concentration ($r = 0.5676$; $r = 0.6302$; $P < 0.01$) and negatively with the ejaculate volume ($r = –0.3456$; $r = –0.2783$, $P < 0.01$). A significant correlation also existed between acid and alkaline phosphatase activity ($r = 0.7043$, $P < 0.01$).

Key words: Polar fox, semen, enzyme, reproductive season

Introduction

Artificial insemination with fresh or frozen-thawed semen has been previously investigated in polar foxes (1-3). An important consideration, prior to insemination, is the selection of a fertile male breeder, which can be determined on the basis of semen analysis. The fertility of a male depends on many exogenous and endogenous factors that may cause disruption within the spermatogenesis and spermiogenesis processes. A semen evaluation should include a percentage of active sperm cells and their type of motion, sperm concentration, and sperm morphology as well as the complex estimation of these factors' influence on fertility (4). The aim of this research was to evaluate the biologic properties of polar fox (*Alopex lagopus* L.) semen at several times during the breeding season.

Materials and methods

Semen was collected from eighteen 1-year-old polar foxes (*Alopex lagopus* L.) kept on a fur farm in Łachowo near Szubin in Kujawsko-Pomorskie Voivodeship. Semen samples were obtained during the period of intensive sexual activity (i.e. February to April). Semen was collected using a manual method 7 times from each individual at 10- to 12-day intervals, yielding a total of 126 ejaculates. For each ejaculate, the pH and volume (cm^3) were measured. Sperm concentration was determined using a hemocytometer and a phase contrast microscope. Sperm cell morphology was determined after staining samples with the *Bydgoska* method. Abnormalities in morphological structure were classified according to Bloom (5). Semen was then centrifuged for 10 min at 8000 × *g*, and total protein (6) concentration was

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determined from the seminal plasma using the method described by Bessey et al. (7). Seminal plasma acid phosphatase and alkaline phosphatase concentrations were determined at the Institute of Animal Reproduction and Food Research of the Polish Academy of Sciences in Olsztyn.

Comparisons between when the semen was collected during the breeding season to selected semen biologic parameters were made using non-parametric one-way analysis of variance (Kruskal-Wallis tests). Correlations between variables were calculated using Spearman correlation coefficients.

Results

Characteristics of ejaculated fox semen obtained during the breeding season are summarized in Table 1. In the ejaculates sampled, the sperm cell concentration averaged 491.77 × 10^6/cm^3, ranging from 73.68 to 799.72 × 10^6/cm^3. The mean volume of the single ejaculate was 0.39 cm^3. The morphological analysis indicated that 89.88% of the sperm cells were normal. Most of the abnormal morphology sperm cells had minor defects, including a single sperm tail loop, loop or bending at the end of a tail, and a loose but regular head. Few sperm (1.84%) had major defects, such as Dag defects and lamellar defects. In general, the biological value of the semen examined can be considered high, although the ejaculates included some morphologically deformed sperm cells (10.11% on average). The protein content in polar fox seminal plasma ranged from 33.23 to 48.91 mg/cm^3. The average seminal plasma acid phosphatase activity was 136.66 U/dm^3 and alkaline phosphatase activity was 10.21 × 10^4 U/dm^3.

Table 1. Characteristics of the polar fox semen during the breeding season (± SD).

Parameters	Semen collection at different periods of the breeding season							
	total n = 126	1 n = 18	2 n = 18	3 n = 18	4 n = 18	5 n = 18	6 n = 18	7 n = 18
Ejaculate volume (cm^3)	0.39 ± 0.26	0.27 ± 0.16	0.32 ± 0.26	0.53 ± 0.35	0.42 ± 0.26	0.28 ± 0.07	0.31 ± 0.18	0.57 ± 0.25
Sperm cell concentration × 10^6/cm^3	491.77 ± 372.12	674.85 ± 367.04	799.72 ± 440.74	554.24 ± 348.55	73.68 ± 256.50	273.54 ± 170.31	490.94 ± 401.27	275.42 ± 121.38
pH	6.12 ± 0.20	6.23 ± 0.20	6.14 ± 0.16	6.13 ± 0.34	6.13 ± 0.15	6.03 ± 0.14	6.17 ± 0.18	6.02 ± 0.07
% normal morphology	89.88 ± 4.40	88.21 ± 4.18	91.09 ± 4.55	90.48 ± 4.93	90.37 ± 3.76	86.59 ± 4.96	90.02 ± 3.72	92.42 ± 1.89
% abnormal morphology (major defects)	1.84 ± 1.31	2.26 ± 0.87	1.70 ± 0.80	1.76 ± 1.12	0.52 ± 0.93	3.33 ± 1.07	2.15 ± 1.29	1.18 ± 1.13
% abnormal morphology (minor defects)	8.27 ± 4.13	9.54 ± 4.13	7.20 ± 4.03	7.78 ± 4.93	9.09 ± 3.72	10.07 ± 4.94	7.83 ± 3.66	6.39 ± 2.22
Total protein concentration [mg/cm^3]	44.14 ± 13.81	45.32 ± 10.30	48.91 ± 9.08	42.67 ± 14.94	42.25±16.29	33.23 ± 13.72	48.51 ± 11.82	47.97 ± 13.53
Acid phosphatase activity [U/dm^3]	136.66 ± 65.27	171.46 ± 46.58	173.04 ± 64.27	116.97 ± 56.92	124.29 ± 59.84	82.11 ± 34.64	165.38 ± 95.31	126.87 ± 38.18
Alkaline phosphatase activity × 10^4 [U/dm^3]	10.21 ± 9.56	16.60 ± 10.67	16.33 ± 11.44	10.58 ± 8.14	6.55 ± 4.58	1.51 ± 3.37	9.64 ± 11.94	8.04 ± 4.32

Discussion

Seminal plasma acid phosphatase is involved in sperm capacitation, acrosome reaction (8), hyperactivation, and zona pellucida binding (9). The average acid phosphatase activity in the polar fox (136.66 U/dm^3) was much lower than in the domestic boar (10) or the wild boar × domestic pig hybrid (11) (346.68 U/dm^3 and 417.17 U/dm^3, respectively). Even in red deer, which are also seasonal breeders, seminal plasma acid phosphatase activity was higher in comparison with polar fox semen: 948.5 U/dm^3 and 294.6 U/dm^3 in white and yellow fractions of the ejaculate, respectively (12).

The main source of the acid phosphatase enzyme in canine and human seminal plasma is the prostatic gland secretion. However, according to Wysocki and Strzeżek (13), there are other sources of this enzyme, like for example epididymis as well as the follicular glands in case of the boar. However, the polar fox has no follicular glands, which implicates that the acid phosphatase is secreted by the prostate and epididymis only. The latter was confirmed also by this study, in which a highly significant relation between activity of the acid and alkaline phosphatase was obtained.

Acid phosphatase activity in polar fox seminal plasma was at the highest concentrations at the beginning of the breeding season, represented by the first and second semen collections. Those results indicate that, in the first phase of the polar fox sexual activity season, the gametes are of high maturity as they derive from the tail of the epididymis. The enzymatic activity measured later during the breeding season gradually decreased and the lowest value was obtained in the fifth ejaculate collection. Reduction in the activity of the acid phosphatase suggests too intensive exploitation of the males, which, as a consequence, results in immaturity of the sperm cells in the semen, originating not only from the tail, but also from the body of the epididymis. Contradictory results were obtained in the seminal plasma, where the acid phosphatase activity had increased by the end of the breeding season. However, full interpretation of this phenomenon is difficult, as we lack data on the morphological defects of the sperm cells connected with the acrosome. Seminal plasma acid phosphatase activity was significantly correlated with sperm cell concentration, which is similar to what has been reported in the wild boar × domestic pig hybrid. Similar to the findings in the current study, Glogowski et al. (10) also demonstrated a similar significant correlation between acid phosphatase activity and total protein content in boar semen. The relation between the acid phosphatase activity and the selected semen parameters is shown in Table 2.

Table 2. Relationship between acid phosphatase and alkaline phosphatase activity and chosen indicators of semen quality.

Indicator of semen quality	Acid phosphatase activity	Alkaline phosphatase activity
Sperm cell concentration	0.5676**	0.6302**
Ejaculate volume	–0.3456**	–0.2783**
% normal morphology	–0.0451	0.0213
% abnormal morphology (major defects)	–0.2078*	–0.2528**
% abnormal morphology (minor defects)	0.0915	0.0494
Total protein content	0.5780**	0.4158**
Acid phosphatase activity	-	0.7043**
Alkaline phosphatase activity	0.7043**	-

* $P < 0.05$, ** $P < 0.01$

Alkaline phosphatase is a main secretion of the epididymis, the organ that plays a crucial role in the sperm cells' maturation (10,14), but the activity of this enzyme was also determined in the cytoplasmic droplets of the sperm cells, which suggests its association with glycogen metabolism in epididymal epithelium, thus supplying the maturing spermatozoa with energy (15). Alkaline phosphatase also participates in producing free semen fructosis, which, after fructolysis, provides the energy indispensable for the sperm cells' motility (16). Alkaline phosphatase activity in seminal plasma is much higher (336-fold) in canine than in human seminal plasma. In our study the activity of alkaline phosphatase enzyme averaged 10.21×10^4 U/dm^3, which was 2.5 times higher than the results obtained by Gobello et al. (17) in canine seminal plasma ($0.5\text{-}4.0 \times 10^4$ U/dm^3). Slightly lower activity of alkaline phosphatase in animal seminal plasma was determined in the boar (1.42×10^4 U/dm^3) (10) as well as in the wild boar × domestic pig crossbreed (2.20×10^4 U/dm^3) (11). The lowest enzyme activity was estimated in seminal plasma of the red deer, with scope of $0.68\text{-}2.47 \times 10^4$ U/dm^3 (12). In our study the activity of alkaline phosphatase was significantly correlated with sperm cells concentration, which corresponds well with the literature data (10-12). This fact, combined with high alkaline phosphatase activity, indicates very good quality of the fox ejaculate analyzed, containing fully mature (as deprived of protoplasmic droplets) sperm cells. In addition, the correlation between both acid and alkaline phosphatase activity and sperm cells concentration as well as ejaculate volume suggests that at the beginning of the breeding season the seminal plasma was derived from the epididymis, whereas at the end of the breeding season it mostly consisted of prostate secretion.

In the current study, polar fox seminal plasma protein content ranged from 33.23 to 48.91 mg/cm^3. Seminal plasma total protein content was unchanged during the breeding season. However, slight differences within seminal plasma total protein may suggest changes in accessory gland secretions. Proteins secreted by the accessory glands are physiologically importantly for both the male (18) and the female (19). Canids (including dogs and foxes) only have one accessory gland (the prostate) (20) and the protein content in the seminal plasma is dependent upon secretions from the epididymises and the prostate gland (21). It is important to note that, out of the 7 semen samples collected at 10- to 12-day intervals, the studied biologic indicators decreased only in the fifth collection. Seasonal changes (winter to spring) relating to increasing temperature and day length may be responsible for this observation.

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