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Short communication

Determination of sperm acrosin activity in the arctic fox (*Alopex lagopus* L.) – using method developed for human spermatozoa

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Abstract

The aim of the study was to adapt a method to determine acrosin activity of human spermatozoa to arctic fox (*Alopex lagopus* L.) spermatozoa. We modified this method by reducing sperm count per sample from $1 \div 10 \times 10^6$ to $25 \div 200 \times 10^3$, incubation time from 180 minutes to 60 minutes, and Triton X-100 concentration in the reaction mixture from 0.01% to 0.005% per 100 cm^3. It has also confirmed that arctic fox seminal plasma is rich in proteinases and their inhibitors. To completely abolish the inhibitory effect of seminal plasma on acrosin activity it is recommended to wash the spermatozoa four times. Benzamidine served an inhibitor of acrosin activity.

Key words: fox, acrosin activity, spermatozoa

Introduction

The sperm acrosome contains a variety of hydrolytic enzymes, of which the largest group are proteinases. Acrosin (EC 3.4.21.10) belongs to serine proteinases, i.e. proteinases with a serine at the active site. Acrosin plays a pivotal role in mammalian fertilization because it catalyzes hydrolysis of the zona pellucida and is involved in the dispersion of the acrosomal matrix and in the binding of spermatozoa to the zona pellucida (Mari et al. 2003). Differences in collection, preparation of samples of semen and sperm acrosin content in different species of mammals, birds and fish provide the basis for adaptation developed by Kennedy et al. (1989) method of determining the activity of this enzyme. Because the fox is the animal most valuable to the fur trade (in addition to mink) and the acrosin plays an indisputable role, we attempted to optimize the method for determination of acrosin activity in this species.

Materials and Methods

The study used semen from eight arctic foxes (*Alopex lagopus* L.) aged between 1 and 3 years, originating from a fur farm in Łachowo near Szubin in the Kujawsko-Pomorskie province. Semen samples

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were collected manually from males once a week. The experiments were carried out with permission from the Local Ethical Commitee in Bydgoszcz (1/2010). Adapting the method developed by Kennedy et al. (1989) relied on researching the effect of the following parameters on acrosin activity: sperm count per sample (from 25 to 400×10^3), incubation time at 25°C (from 15 to 90 min), Triton X-100 concentration (from 0 to 0.05%), seminal plasma added (plasma dilution range of 10- to 10^5-fold), and benzamidine concentration. The date were analysed using STATISTICA 8.0 program (StatSoft, USA).

Results and Discussion

The range of reproductive cells counts in foxes was much lower than the ranges determined in humans: $2 \div 10 \times 10^6$ spermatozoa/ml (Kennedy et al. 1989) and even the turkey: $0.25 \div 10 \times 10^5$ spermatozoa/ml (Glogowski et al. 2001). A linear correlation ($r^2 = 0.998$) was found between sperm concentration (from 25,000 to 200,000) and the activity of the enzyme under study. The mean number of spermatozoa per sample was set at 200,000 because linearity was lost when this figure was exceeded. It is probable that with a greater number of spermatozoa per sample the inhibitors present in seminal plasma interfere with the enzyme activity.

Without question, acrosin activity is influenced by the duration of proacrosin incubation. For a 60-minute incubation the measured activity (12.7 mU/mln sperm) did not differ significantly from the activity measured after 1.5 hours (14.8 mU/mln sperm). Our study shows that the incubation for 1 h is the optimum time for proacrosin conversion into acrosin. The time of proenzyme activation set in our study was similar to the values determined for boar (Glogowski et al. 1998) and turkey spermatozoa (Glogowski et al. 2001).

Triton X-100 surfactant was used for cell membrane lysis. The highest (but non-significant) activity was noted in the samples containing 0.005% of this surfactant, which is why this reaction mixture was used for further optimization of the method. The noticeable differences in the content of Triton X-100 in the reaction mixture for different animal species: boar – 0.005% (Glogowski et al. 1998) and turkey – 0.5% (Glogowski et al. 2001) suggest that sperm membranes vary in their susceptibility to this detergent.

Our study demonstrated that arctic fox seminal plasma is rich in both proteinases and their inhibitors (Stasiak and Janicki 2007). Supplementation of the samples with a 10-fold diluted plasma caused the increase in acrosin activity (5.05 mU/mln sperm). Unlike boar spermatozoa, we obtained a 50% inhibition of acrosin activity when seminal plasma was diluted 10,000-fold rather than 1,000-fold (Glogowski et al. 1998). To completely abolish the inhibitory effect of seminal plasma on acrosin activity, we considered it necessary to increase the number of sperm washes.

One of the compounds capable of inhibiting acrosin activity is benzamidine. When added to the samples at a concentration of 0.1 mM, this inhibitor reduced acrosin activity to 0.881 mU/mln sperm. Meanwhile, supplementation of the incubated samples with benzamidine at a concentration of 10 mM caused an approximately 96% decline in the activity of this protease. Our findings are supported by Glogowski et al. (2001) who reduced acrosin activity in turkey spermatozoa by approx. 99% when supplementing the samples with benzamidine at a concentration of 10 mM.

In summary the results obtained in the present study show that the determination of acrosin activity in arctic fox spermatozoa using the method should be optimized.

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