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Short communication

Effect of dialysis of dog semen on sperm characteristics and some biochemical components of seminal plasma

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Abstract

The aim of this study was to investigate the effect of dog semen dialysis on sperm characteristics and some biochemical components of seminal plasma. Whole ejaculates were dialyzed against Tris-citrate-fructose extender for a 5 h period at room temperature (using semi-permeable cellulose tubing of 12-14 kDa molecular weight cut-off). It has been demonstrated that the long-term dialysis of dog semen causes a significant decrease in sperm quality parameters and disrupts the biochemical properties of seminal plasma. This procedure requires further improvement.

Key words: dog, semen, dialysis, seminal plasma

Introduction

The effects of seminal plasma on sperm during dog semen preservation are still controversial. It is now recommended that the seminal plasma should be removed by centrifugation at the initial stages of preservation. This may, however, result in the loss of some of the seminal plasma components, e.g. antioxidants, which have a protective effect on spermatozoa properties (Koderle et al. 2011).

One possibility of improving the efficiency of the whole ejaculate in preservation technology is the dialysis procedure. Earlier research indicated a positive effect of 5 h dialysis of boar semen on sperm quality parameters (Fraser et al. 2007) and therefore this study investigates the effect of dog semen dialysis on sperm characteristics and some biochemical components of seminal plasma.

Materials and Methods

Whole ejaculates from six mixed-breed dogs (aged 2-8 years) were collected once weekly over a period of 4 weeks. Aliquots of freshly ejaculated semen were separated for evaluation prior to dialysis. The remaining semen samples were dialyzed using semi-permeable cellulose tubing of 12-14 kDa molecular weight cut-off (Visking Dialysis Tubing, Serva Electrophoresis, Germany) against standard Tris-based extender, (1:40; semen : dialysate) (250 mM Tris (hydroxymethyl) aminomethane, 62 mM fructose, 60 mM sodium citrate monohydrate, pH 6.4) for 5 h at room temperature. Spermatozoa from semen samples (non-dialyzed and dialyzed) were evaluated by microscopic analysis of total motility (TM) and fluorescent assessments of plasma membrane integrity (PMI) and mitochondrial function (MF) using

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Table 1. Effect of dog semen dialysis on sperm characteristics and components of seminal plasma (n=24).

Semen treatment	Sperm characteristics			Seminal plasma					
							Low-molecular weight antioxidants		
	TM (%)	PMI (%)	MF (%)	TPC (mg/ml)	Zn^{2+} (mM)	SH (mM)	GSH +GSSH (mM)	ERG (μM)	ASC (μM)
Non-dialyzed	81.06[a] ± 1.22	78.57[a] ± 3.19	83.80[a] ± 2.41	36.5[a] ± 3.30	1.38[a] ± 0.19	4.95 ± 0.51	2.86 ± 0.26	0.91 ± 0.04	119.23[a] ± 23.0
Dialyzed	11.87[b] ± 4.11	29.20[b] ± 9.29	40.33[b] ± 10.93	50.9[b] ± 2.52	0.57[b] ± 0.11	5.70 ± 0.47	3.62 ± 0.28	1.36 ± 0.04	56.77[b] ± 4.54

Note: different letters indicate significant differences at $p \leq 0.05$

methods proposed by Strzeżek and Fraser (2009). The seminal plasma (non-dialyzed and dialyzed) was analyzed for total protein content (TPC) measured using the biuret method and Zn^{2+} content using the method of Lampugnani and Maccheroni (1984). Also, free thiol group (SH) content and the concentrations of low molecular weight antioxidants in the seminal plasma, such as L-glutathione (GSH+GSSG), L-ergothioneine (ERG) and L-ascorbic acid (ASC) were determined according to the methods proposed by Strzeżek et al. (2009).

Results and Discussion

The pH level of ejaculate changed from 6.45 ± 0.06 (non-dialyzed) to 7.1 ± 0.17 (dialyzed). The process of dialysis resulted in a significant decrease in sperm quality parameters, such as TM, PMI and MF (Table 1). Also, a strong agglutination of spermatozoa was detected (data not shown). The significant increase of TPC content in seminal plasma may have been caused by the interference of the biuret method and the reducing factors (SH, GSH+GSSH and ERG), which also increased after dialysis (Buxbaum 2011). Moreover, the increase of TPC may suggest that after dialysis of dog semen the structure of the seminal plasma proteins undergoes conforming changes. This may also have been a cause of the disruption of the stability of Zn^{2+} ion complexes with protein components of seminal plasma, and the release of these ions into the dialysis environment. The significance of Zn^{2+} ions which originated in dogs mainly from the prostate, for the functional properties of dog spermatozoa must be emphasized (Mogielnicka-Brzozowska et al. 2012). Additionally, the significant decrease of ASC content may have been a cause of the observed functional changes of dog spermatozoa, since the positive effect of ASC on the stability of sperm plasma membranes has been demonstrated (Ceylan and Serin, 2007). In summary, the long-term dialysis procedure of dog semen prior to the subsequent stages of preservation is not recommended.

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