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Short communication

Effect of different egg yolk sources on dog semen quality following cryopreservation

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Abstract

The aim of this study was to compare the cryoprotective effects of egg yolk from different avian species (hen, goose and quail) on post-thaw quality of dog semen. Total motility (TMOT) and progressive motility (PMOT) of frozen-thawed spermatozoa were not significantly differed among the extenders, but were higher in the quail-egg yolk based extender compared with extender containing hen or goose egg yolk. It was found that post-thaw sperm motion parameters, velocity VCL and ALH, were significantly higher in the quail-egg yolk based extender. No marked differences in post-thaw sperm plasma membrane integrity (PMI) and mitochondrial membrane potential (MMP) were observed among the extenders. In conclusion, the results of the present study suggest that goose or quail egg yolk is a suitable alternative to hen egg yolk for the cryopreservation of dog semen.

Key words: dog, semen, spermatozoa, extender, egg yolk

Introduction

Due to the easy availability of hen egg yolk (HEY), it has been commonly used as a cryoprotective additive for freezing-thawing of semen. Moreover, egg yolk from different avian species has been successfully used as a protective additive for the cryopreservation of semen from various animal species (Santiago-Moreno et al. 2008). However, there are limited data on the protective effects of different sources of egg yolk on dog semen quality following cryopreservation. The aim of this study was to compare the cryoprotective effects of egg yolk from different avian species on the post-thaw quality of dog semen.

Materials and Methods

Sperm-rich fractions of ejaculates were collected weekly for a 4-week period from four dogs of mixed-breed (aged 5-10 years). Semen samples were pooled, centrifuged (700 x g, 5 min) and the sperm pellets were re-suspended in a standard Tris-citrate-fructose (TCF) extender. Following equilibration for 30 min at room

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Table 1. Effect of different egg yolk sources on post-thaw quality of dog semen. Values are expressed as means ± SEM.

Extender type	Motility parameters								PMI (%)	MMP (%)
	TMOT (%)	PMOT (%)	VAP (μm/s)	VSL (μm/s)	VCL (μm/s)	ALH (μm)	BCF (Hz)	LIN (%)		
TCF-HEN	66.14±1.09	45.30±1.41	121.41±1.29	108.72±1.51	159.84^{a}±1.94	5.39^{a}±0.12	12.27±0.64	70.22^{a}±0.74	49.22±1.44	44.62±1.09
TCF - GOOSE	68.84±1.44	45.08±1.70	119.49±1.97	104.50±2.14	162.49^{a}±2.82	5.75^{a}±0.13	12.61±0.94	66.68^{ab}±0.94	50.13±1.87	43.83±1.38
TCF-QUAIL	69.95±1.95	49.67±2.22	125.13±2.73	110.22±2.59	174.82^{b}±3.84	6.27^{b}±0.16	14.98±1.10	65.22^{b}±1.16	50.35±1.64	45.72±1.67

Values within the same column with different superscripts (a, b) are significant at $p<0.05$. TMOT – total motility; PMOT – progressive motility, VAP – velocity average path, VSL – velocity straight line, VCL – velocity curvilinear, ALH – amplitude of lateral head displacement, BCF – beat cross frequency, LIN – linearity; PMI – plasma membrane integrity, MMP – mitochondrial membrane potential

temperature, the semen samples were cooled for 90 min at 5°C, before being divided into three equal portions. Each portion was diluted (1:1) with 5.0 ml TCF-glycerol extender (0.8 ml glycerol and 0.2 ml Orvus Es Paste) comprising 4.0 ml HEY (TCF-HEY), goose egg yolk (TCF-GOOSE) or quail egg yolk (TCF-QUAIL). The final concentration of egg yolk in each extender was 20 %, while the final concentrations of glycerol and Orvus Es Paste were 4% and 1%, respectively. All TCF-egg yolk extended semen samples were cooled for 15 min at 5°C, before being packaged in 0.25-ml straws and frozen in liquid nitrogen vapor for 10 min. The samples were thawed in a water bath for 5 sec at 70°C. Post-thaw semen was evaluated for motility parameters, using the computer-assisted sperm analysis system (CASA) (HTM-IVOS ver. 12.3, Hamilton Thorne Biosciences, MA USA), and fluorescent assessments of plasma membrane integrity (PMI) and mitochondrial membrane potential (MMP), according to a previously described method (Strzeżek et al. 2015). The data were analyzed using the Statistica software package, version 13.0 (StatSoft).

Results and Discussion

No significant differences were observed in post-thaw sperm motility (TMOT), progressive motility (PMOT), velocity average path velocity (VAP), velocity straight line velocity (VSL) and beat cross frequency (BCF) among the extenders (Table 1). However, the extender containing quail egg yolk exhibited significantly higher ($p<0.05$) post-thaw values for velocity curvilinear (VCL) and amplitude of lateral head displacement (ALH) compared with the other egg yolk-based extenders (Table 1). In addition, TCF-QUAIL extender showed significantly lower ($p<0.05$) post-thaw values for linearity (LIN) compared with the TCF-HEY extender. No significant differences ($p>0.05$) in post-thaw sperm plasma membrane integrity (PMI) and mitochondrial membrane potential (MMP) were observed among the extenders (Table 1).

It has been suggested that phospholipids, cholesterol and low-density lipoproteins (LDLs) in egg yolk provide protective effects to sperm against cold shock during the freezing-thawing process (Kulaksiz et al. 2010). However, there are variations in the composition of egg yolk from different avian species, particularly in terms of the content of cholesterol, fatty acids and phospholipids (Santiago-Moreno et al. 2008). Besides hen egg yolk, it has been suggested that the use of egg yolk from different avian species could have varying protective effects on the cryo-survival of mammalian spermatozoa (Bathgate et al. 2006). However, the findings of the present study show that the components of egg yolk from different avian species (hen, goose and quail) seem to provide similar protective effects on dog sperm functions following cryopreservation. It should be noted that marked differences in the fatty acid composition and lipid class ratios in spermatozoa among various animal species are important factors affecting sperm freezability (Parks and Lynch 1992). Also, it should be emphasized that differences in the cholesterol content in the membranes of spermatozoa influence their susceptibility to cold shock (Kulaksiz et al. 2010). Moreover, species-specific differences in the sperm

membrane composition and components of avian egg yolk have been shown to affect the sperm cryo-survival (Santiago-Moreno et al. 2008).

For example, Su et al. (2008) reported that better post-thaw sperm motility and viability were achieved when bull spermatozoa were cryopreserved in an extender containing egg yolk from chicken or goose compared with that containing either duck or quail egg yolk. Furthermore, Trimeche et al. (1997) reported that the use of quail egg yolk as an extender component had a better effect on the post-thaw motility of donkey spermatozoa compared with chicken egg yolk. In another study, Bathgate et al. (2006) demonstrated that immediately after post thaw the motility of boar spermatozoa was higher in extender containing chicken egg yolk than in quail egg yolk; however, post-thaw motility did not differ between the egg yolk-based extenders after 3 h or 6 h post-thaw incubation.

The significant increase in post-thaw values of VCL and ALH accompanied by the decrease of LIN in an extender containing quail egg yolk have been described as indicators of hyperactivated motility patterns (Rijsselaere et al. 2012). In the current study, no significant differences in post-thaw TMOT and PMOT were observed among the extenders; however, sperm frozen-thawed in the quail-egg yolk based extender seemed to exhibit hyperactivated motility patterns or increased activity, which might be beneficial in reproductive techniques.

The results of the present study suggest that goose or quail egg yolk is a suitable alternative to the hen egg yolk for the cryopreservation of dog semen. However, further studies are needed to elucidate the potential benefits of the various components of egg yolk from different avian species on the post-thaw quality of dog semen.

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