Effect of Addition of Orvus ES Paste to Frozen Canine Semen Extender on Sperm Acrosomes

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ABSTRACT. Using the triple-stain technique, we investigated whether sperm acrosomes in frozen canine semen were protected during freezing and thawing by addition of a surfactant, Orvus ES Paste (OEP), to the extender. Acrosomes were clearly shown to be protected by the addition of OEP to the extender when compared with those in sperm frozen without OEP addition ($p < 0.05$).—**KEY WORDS:** acrosome, canine, Orvus ES Paste.

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A high percentage of boar sperm are damaged by freezing. Graham *et al.* [1] demonstrated that adding a surfactant, Orvus ES Paste (OEP), to the extender protects acrosomes, making frozen boar semen available for practical use. By electron microscopy, Toyama and Itoh [7] showed that acrosomes are maintained in a normal state by adding OEP to the extender used to freeze boar semen. Kato *et al.* [2] obtained a similar result using sodium laurylsulfate (1.2–1.6 mg/ml, SLS), which is the major ingredient of OEP.

Similar to boar sperm, canine sperm has a low resistance to freezing, and no practical procedure for use of frozen semen has been developed yet. In 1993, Nöthling and Volkmann [3] initially added 0.5% OEP to frozen canine semen. However, they did not discuss the effectiveness of adding OEP to frozen canine semen. Later in 1997 and 1999, Rota *et al.* [4, 5] reported that adding 0.5% OEP to the extender of frozen canine semen increases its effectiveness. We also investigated various concentrations of OEP added to the extender of frozen canine semen, and their effectiveness. The result showed that addition of 0.5–1.0% OEP improves the sperm motility after thawing and maintains the motility for a prolonged time after thawing [8]. The reason for these effects may be that OEP protects acrosomes, as Toyama and Itoh [7] showed in boar sperm.

We therefore, added OEP at a final concentration of 0.75% to Tris-fructose-citrate extender containing 20% (v/v) egg yolk (EYT-FC), which was used by Rota *et al.* [4, 5] as an extender for frozen canine semen, and investigated whether acrosomes were protected using the triple-stain technique (TST) reported by Talbot and Chacon [6].

The five dogs used in the experiments were 2–6 year-old beagles with copulation ability and fertility. The semen samples, collected by digital manipulation, were divided into three fractions. Semen volume, and the number, motility, and viability of sperm of the 2nd fraction were determined by methods previously described [9]. After examination, semen was subjected to the first dilution with EYT-FC, and was then divided into two fractions. In the second dilution, one fraction was diluted with the secondary extender containing OEP, and the other fraction was diluted

with the secondary extender without OEP addition. Both primary and secondary dilutions were performed at room temperature (20°C). The final sperm concentration was adjusted to 1×10^8 /ml, and 0.5 ml straws were used. The final glycerol concentration was adjusted to 7%. Semen was frozen using a conventional freezer with the freezing temperature conditions and method previously described [8].

For the examination of semen quality after thawing, semen was thawed in warm water at 37°C for 45 sec one week or more after freezing. The presence or absence of acrosomes in viable and dead sperm was observed by TST. Specimens from all five experimental dogs were examined once.

The semen quality at collection, and results obtained by TST are shown in Table 1.

The sperm motility in the five dogs was 93.0 ± 1.2 (SE) on average, and the sperm viability was $93.0 \pm 1.2\%$. Of viable sperm observed by TST, $68.0 \pm 3.3\%$ contained acrosomes.

The semen quality and presence or absence of acrosomes after thawing are shown in Table 2.

When OEP was added, sperm motility was $27.0 \pm 4.2\%$ on average, and that in semen without OEP addition was $17.0 \pm 3.4\%$ on average, showing the superiority of the OEP-supplemented group. Sperm viability was $59.7 \pm 7.1\%$ on average and $48.5 \pm 4.9\%$ on average in the OEP-supplemented group and in the group without OEP addition, respectively, clearly showing that the viability was higher in the OEP-supplemented group ($p < 0.05$). On examination by TST, the ratio of viable sperm containing acrosomes was $42.8 \pm 10.0\%$ on average in the OEP-supplemented group, and $10.8 \pm 3.7\%$ on average in the group without OEP addition, again showing the superiority of the OEP-supplemented group ($p < 0.05$).

In this study, the mean sperm viability at semen collection was 92.3%, but the viability decreased by approximately 20% to 72.2% when observed by TST. A similar result was obtained in the frozen semen without OEP addition. Sperm viability may have been reduced by the multiple centrifugation procedures required in TST. In

Table 1. Semen quality and presence or absence of acrosomes at semen collection

Dog No.	Semen quality (%)		Presence or absence of acrosomes (%)			
	Motility	Viability	Viable	sperm	Dead	sperm
			+	–	+	–
B 253	95	90.7	65	9	24	2
B 266	90	88.9	71	0	18	11
B 267	95	94.6	74	6	7	13
B 273	90	90.7	72	3	17	8
B 275	95	96.8	58	3	32	7
Mean	93.0	92.3	68.0	4.2	19.6	8.2
± SE	1.2	1.5	3.3	1.7	4.6	2.1

Table 2. Semen quality and presence or absence of acrosomes after thawing

Dog No.	Semen quality (%)				Presence or absence of acrosomes (%)							
	OEP (+)		OEP (–)		OEP (+)				OEP (–)			
	Motility	Viability	Motility	Viability	Viable	sperm	Dead	sperm	Viable	sperm	Dead	sperm
					+	–	+	–	+	–	+	–
B 253	20	41.3	10	35.4	33	12	25	30	14	9	22	55
B 266	30	48.9	25	50.2	21	29	18	32	3	6	4	87
B 267	25	73.0	20	60.6	58	15	2	17	9	22	14	55
B 273	40	62.6	10	42.3	33	20	19	28	6	34	13	47
B 275	20	72.7	20	54.2	69	5	23	3	22	13	13	52
Mean	27.0	59.7 ^{a)}	17.0	48.5	42.8 ^{b)}	16.2	17.4	22.0	10.8	16.8	13.2	59.2
± SE	4.2	7.1	3.4	4.9	10.0	4.5	4.5	6.1	3.7	5.7	3.2	7.9

a), b): Significantly different from OEP (–) at $p < 0.05$.

contrast, in the frozen semen to which OEP was added, the viability of spermatozoa was not decreased on TST.

In this study, we showed that acrosomes are protected by addition of OEP to the extender of frozen canine semen, which increased the content of protein and SH groups in the semen and protected acrosomes, thereby reducing the harmful effects of hydrogen peroxide on sperm [2,7].

REFERENCES

- Graham, E.F., Rajamannan, A.H.J., Schmehl, M.K.L., Maki-Laurila, M. and Bower, R.E. 1971. *A. I. Digest* 19: 12–14.
- Kato, S., Miyano, T., Nanjo, I., Yasui, T. and Kanda, S. 1990. *Jpn. J. Anim. Reprod.* 36: 26–30.
- Nöthling, J.O. and Volkmann, D.H. 1993. *J. Reprod. Fertil.* 47: 329–333.
- Rota, A., Ström, B., Linde-Forsberg, C. and Rodriguez-Martinez. 1997. *Theriogenology* 47: 1093–1101.
- Rota, A., Igner-Ouda, M., Verstegen, J. and Linde-Forsberg, C. 1999. *Theriogenology* 51: 1045–1058.
- Talbot, P. and Chacon, R.S. 1981. *J. Exp. Zool.* 215: 201–208.
- Toyama, Y. and Itoh, Y. 1995. *J. Reprod. Dev.* 41: j9–j13.
- Tsutsui, T., Hase, M., Hori, T. and Kawakami, E. 2000. *J. Vet. Med. Sci.* 62: 533–535.
- Tsutsui, T., Tezuka, T., Shimizu, T., Murao, I., Kawakami, E. and Ogasa, A. 1988. *Jpn. J. Vet. Sci.* 50: 193–198.