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The fertility of canine spermatozoa cryopreserved with skim milk based extender

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Cryopreservation of canine spermatozoa provides potential exchange of genetic materials, and may lead to improvement in the breeding management programs used in working dogs. Although the conventional cryoprotectant, egg yolk (EY), for canine semen has been successfully used, transportation of frozen semen with EY has been compromised when avian influenza was prevalent. We previously reported that skim milk (SM) is an effective cryoprotectant for cryopreservation of canine spermatozoa instead of EY. In this study, the fertilizing ability of canine spermatozoa frozen with SM by transcervical insemination (TCI) was evaluated for practical use. Collected ejaculates were cryopreserved with SM based-extender (30 mg/ml SM, 0.2 M raffinose pentahydrate and 7% [v/v] glycerol) or EY based-extender (20% [v/v] EY, 24 mg/ml Tris, 14 mg/ml citric acid monohydrate, 0.8 mg/ml glucose and 7% [v/v] glycerol). After thawing, the semen samples were inseminated to 24 and 94 bitches by TCI in SM and EY groups, respectively. To estimate the LH surge, the plasma progesterone concentrations of the bitches were measured daily by enzyme-linked fluorescent assay (SV-5010, Spotchem Vidas; Arkray, Kyoto, Japan). The day when the plasma concentration of progesterone exceeded 2 ng/ml was estimated as the occurrence of the LH surge (defined as Day 0). TCI with frozen-thawed semen were performed 1 to 4 times on Day 2–9. Data on delivery rates and litter sizes were compared using chi-square test with Yates' continuity correction and one-way analysis of variance (ANOVA) with Fisher protected least significant difference, respectively. Differences were considered significant at a level of P < 0.05. The delivery rates and litter size in SM group (54.2% and 3.8, respectively) were not significantly different compared with those in EY group (46.8% and 4.4, respectively). However, when bitches were inseminated with cryopreserved spermatozoa only once (single TCI) on Day 4–8, the delivery rate was higher in SM group (63.6%) than in EY group (32.1%). In SM group, single TCI on Day 5 lead to higher delivery rate and litter size than any other days post LH surge (83.3% and 5.0 vs. 0.0-50.0% and 0.0-1.0, respectively), although those on Day 6 was higher comparing with any other days in EY group (42.9% and 5.0 vs. 0.0-41.7% and 0.0-2.8, respectively). These findings suggest that skim milk is a suitable alternative to egg yolk for cryopreservation of canine spermatozoa, and the suitable timing for insemination might be 5 day post LH surge.