IGF-I Improves Mitochondrial Membrane Potential during Hypothermic Storage of Canine Spermatozoa

Sang-Min SHIN1), Suhee KIM2), Jin-Gi HONG1) and Yong-Jun KIM1)*

1)Department of Veterinary Obstetrics and Theriogenology, College of Veterinary Medicine, Chonbuk National University, Jeonju, Jeonbuk 561–756, Republic of Korea
2)Department of Biochemistry, School of Dentistry, Chonnam National University, Gwangju 500–757, Republic of Korea

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ABSTRACT. The aim of study was to evaluate the effects of insulin-like growth factor I (IGF-I) on canine sperm function during cooled and freeze-thaw storage. Extenders supplemented with different IGF-I concentrations (0, 100 and 200 ng/ml) were added to canine spermatozoa, and the sperm samples were stored at 4°C for 48 hr or freeze-thawed. Sperm motility, morphology, plasma-membrane integrity (PMI) and mitochondrial membrane potential (MMP) were evaluated. IGF-I had no effect on PMI or morphology during cooling and freeze-thawing. However, IGF-I alleviated the reduction in progressive motility and MMP caused by cooled storage and led to an improvement in MMP after freeze-thawing. In conclusion, IGF-I can be helpful to maintain progressive motility of canine spermatozoa during hypothermic storage via increased MMP.

KEY WORDS: canine spermatozoa, hypothermic storage, insulin-like growth factor I (IGF-I), mitochondrial membrane potential (MMP).


Dogs are not only companions but also valuable animals capable of representing human disease, because of their pathological similarities with humans [9]. Thus, strategies for long-term storage of canine spermatozoa are promising for the future. Various additives have been used to preserve functional spermatozoa longer [5]. Growth factors are potential candidates to maintain sperm function as an energy source for spermatozoa. In particular, insulin-like growth factor I (IGF-I) improves the quality of mammalian spermatozoa [1, 5, 12, 14]. However, no study has investigated the effect of IGF-I on canine spermatozoa. Therefore, the goal of this study was to determine whether IGF-I plays a beneficial role in canine spermatozoa during hypothermic storage.

Twelve ejaculates were collected from six beagles, and spermatozoa were diluted with an extender (20% [v/v] egg yolk, 5% [v/v] glycerol and 0.5% [v/v] Equex STM paste in a Tris diluent) containing different IGF-I concentrations (0, 100 or 200 ng/ml). The samples were cooled for 0, 12, 24, 36 and 48 hr or freeze-thawed using a standard cryopreservation protocol [10]. Progressive motility [11], morphology [7], plasma membrane integrity (PMI) [4] and mitochondrial membrane potential (MMP) [3] were evaluated. In addition, PMI and MMP of fresh spermatozoa were evaluated after subjecting the samples to different IGF-I concentrations. Sperm PMI and MMP were analyzed using a FACScalibur flow cytometer (Becton Dickinson, San José, CA, U.S.A.) and Cell Quest Pro software (Becton Dickinson) after 6-CFDA/propidium iodide (PI) and JC-1 staining, respectively. CFDA+/PI− and JC-1 aggregate-forming spermatozoa were considered to have intact plasma membranes and a high MMP, respectively.

Statistical analysis was performed using SPSS software (SPSS, Inc., Chicago, IL, U.S.A.). One-way repeated-measures analysis of variance or Friedman test was used according to the normality of the distribution. Statistical significance was set at P<0.05, and all data were presented as mean ± standard error.

IGF-I had no effect on PMI or MMP of fresh spermatozoa (Fig. 1). Progressive motility of spermatozoa decreased in the IGF-I-free condition at 48 hr of cooled storage (P<0.05) (Fig. 2A). However, progressive motility of spermatozoa under IGF-I treatment was not different following 48 hr of cooled storage (Fig. 2A), indicating that progressive motility of spermatozoa can be maintained by IGF-I for long periods of cooled storage. Moreover, IGF-I treatment resulted in increased progressive motility of cooled spermatozoa compared to 0 ng/ml IGF-I at 36 and 48 hr for 100 ng/ml IGF-I and at 36 hr for 200 ng/ml IGF-I (P<0.05) (Fig. 2A).

Cooling for 48 hr did not affect sperm morphology or PMI, and IGF-I did not influence morphology or the PMI of cooled spermatozoa (Fig. 2B and 2C).

Sperm MMP decreased gradually beginning at 12 hr during cooled storage in the IGF-I-free condition (P<0.05) (Fig. 2D). However, MMP of sperm treated with 100 and 200 ng/ml IGF-I increased for 12 hr and 24 hr of cooled storage compared to that at 0 hr, respectively (P<0.05) and was maintained by 36 hr of cooled storage (Fig. 2D). Although sperm MMP decreased at 48 hr of cooled storage compared to that at 0 hr despite IGF-I treatment (P<0.05), the reduction in sperm MMP at 48 hr of cooled storage was mitigated...
by IGF-I, showing increase in high MMP in IGF-I treated samples compared to that in IGF-I-free samples at 48 hr of cooled storage ($P<0.05$) (Fig. 2D).

Progressive motility, morphology and PMI were not different among freeze-thawed spermatozoa in different IGF-I concentrations, whereas the percentage of freeze-thawed spermatozoa with a high MMP increased following IGF-I treatment ($P<0.005$) (Fig. 3).

This study is the first to investigate the effect of IGF-I on canine sperm function during hypothermic storage. IGF-I alleviated sperm damage caused by cooling and freezing by maintaining progressive motility of spermatozoa and preventing a reduction in sperm MMP during hypothermic storage. The IGF-I receptor (IGF-IR) signaling pathway mediated by IGF-I may be involved in enhanced canine sperm function. Specific IGF-IRs have been demonstrated in human [8] and bovine [1] spermatozoa, suggesting a possible role of IGF-I as a regulator of sperm function [5]. As the presence of IGF-IR and IGF-I in sperm and semen and the ability of IGF-I to stimulate sperm motility have been identified [1], a relationship between the IGF system and fertilization has been suggested.

Although no reports have identified IGF-IR in canine spermatozoa, our study indirectly shows the presence of an IGF-IR in canine spermatozoa via the IGF-I effect. IGF-I stimulated MMP and motility of hypothermically stored canine spermatozoa in our study. The possible mechanism of how IGF-I maintains motility and MMP is assumed to be through energy metabolism [1], antioxidant effects [13] and high intracellular calcium level by increased ion transport...
In contrast, activation of cellular metabolism by IGF-I may also be related to the generation of free radicals [6]. In our study, the 100 and 200 ng/ml IGF-I concentrations were optimal level and had a positive effect without toxicity to canine spermatozoa. We cannot clearly state the role of IGF-1 in membrane stability [5] of canine spermatozoa, because of less damage to canine sperm PMI during cooling. Overall, the IGF-IR signaling cascade may be a clue to identify molecular mechanisms regulating motility and membrane integrity of canine spermatozoa.

Our results suggest that IGF-I is an effective supplement to improve canine sperm quality for longer periods of cooling and freeze-thawing. IGF-I may enhance canine sperm fertilizing ability by maintaining motility and MMP and preventing a decrease in sperm longevity during hypothermic storage.

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