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Effect of extracellular adenosine 5′-triphosphate on cryopreserved epididymal cat sperm intracellular ATP concentration, sperm quality, and *in vitro* fertilizing ability

Paweena Thuwanut [a,b], Nlin Arya [c], Pierre Comizzoli [b], Kaywalee Chatdarong [a,*]

[a] *Department of Obstetrics, Gynaecology and Reproduction, Faculty of Veterinary Science, Chulalongkorn University, Bangkok, Thailand*
[b] *Center for Species Survival, Smithsonian Conservation Biology Institute, National Zoological Park, Washington DC, USA*
[c] *Department of Preclinic and Applied Animal Science, Faculty of Veterinary Science, Mahidol University, Nakhon Pathom, Thailand*

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ABSTRACT

Intracellular adenosine 5′-triphosphate (ATP) is essential for supporting sperm function in the fertilization process. During cryopreservation, damage of sperm mitochondrial membrane usually leads to compromised production of intracellular ATP. Recently, extracellular ATP (ATPe) was introduced as a potent activator of sperm motility and fertilizing ability. This study aimed to evaluate (1) levels of intracellular ATP in frozen–thawed epididymal cat sperm after incubation with ATPe and (2) effects of ATPe on epididymal cat sperm parameters after freezing and thawing. Eighteen male cats were included. For each replicate, epididymal sperm from two cats were pooled to one sample (N = 9). Each pooled sample was cryopreserved with the Tris–egg yolk extender into three straws. After thawing, the first and second straws were incubated with 0-, 1.0-, or 2.5-mM ATPe for 10 minutes and evaluated for sperm quality at 10 minutes, 1, 3, and 6 hours after thawing and fertilizing ability. The third straw was evaluated for intracellular ATP concentration in control and with 2.5-mM ATPe treatment. Higher concentration of intracellular sperm ATP was observed in the samples treated with 2.5-mM ATPe compared to the controls (0.339 ± 0.06 μg/2×10^6 sperm vs. 0.002 ± 0.003 μg/2×10^6 sperm, $P \leq 0.05$). In addition, incubation with 2.5-mM ATPe for 10 minutes promoted sperm motility (56.7 ± 5.0 vs. 53.3 ± 4.4%, $P \leq 0.05$) and progressive motility (3.1 ± 0.2 vs. 2.8 ± 0.4, $P \leq 0.05$), mitochondrial membrane potential (36.4 ± 5.5 vs. 28.7 ± 4.8%, $P \leq 0.05$), and blastocyst rate (36.1 ± 7.0 and 28.8 ± 7.4%, $P \leq 0.05$) compared with the controls. In contrast, ATPe remarkably interfered acrosome integrity after 6 hours of postthawed incubation. In sum, the present finding that optimal incubation time of postthaw epididymal cat sperm under proper ATPe condition might constitute a rationale for the studies on other endangered wild felids regarding sperm quality and embryo development.

1. Introduction

Intracellular adenosine 5′-triphosphate (ATP) is the main energy support for sperm functions i.e., sperm motility in the female reproductive tract, sperm hyperactivation, and oocyte penetration [1,2]. In mammalian sperm, the principal cellular ATP production is generated in

* Corresponding author. Tel.: +66 2 218 9644; fax: +66 2 252 0738.
E-mail address: ckaywale@yahoo.com (K. Chatdarong).

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mitochondria *via* glycolysis and/or phosphorylation pathway [3]. Adenosine 5′-triphosphate is a product of a metabolic pathway in the sperm mitochondria (present in the midpiece) that is then delivered to the flagellum for sperm motility [4,5].

Sperm cryopreservation, one of the assisted reproductive techniques, has been routinely used as fertility insurance in various species for decades. However, cryopreservation procedures have been reported as causes of the damage to sperm cells including changes in sperm morphology, disruption of sperm plasma and mitochondrial membrane, loss of sperm motility, and acrosome damage [6,7]. The intact of mitochondrial membrane is necessary for ATP generation which is transferred to microtubules leading to sperm motility [6]. In addition, sperm mitochondria function has been accepted as a major role in a number of infertility or subfertility cases, both under experimental and clinical situations [1]. Our previous study in the domestic cats found that the mitochondrial membrane potential was remarkably changed after the cryopreservation process indicating to the cryoinjury susceptibility [7] which is similar to other mammal species: stallions [8], boars [9], and cheetahs [3]. Hence, impaired sperm quality and impaired sperm mitochondria function during the cryopreservation process related to ATP production might be one the of crucial factors altered infertility or subfertility in the felids.

In recent years, a pharmacologic agent, extracellular adenosine 5′-triphosphate (ATPe), was introduced to the study on couples with infertility [10,11], rat epididymal sperm [5], and mouse [12,13] and bovine sperm [14]. The role of ATPe pathway has been investigated. The ATPe activated purinergic receptor (P_2 purinergic receptor) at the sperm cell surface leading to elevation of the intracellular calcium, sperm acrosomal exocytosis, and hyperactivation [14]. In addition, the other studies have been indicated that ATPe activated cAMP signal transduction pathway which elevated mitochondrial calcium level; then, the calcium-dependent dehydrogenases involved in the Krebs cycle was increased. Thus, ATP was generated and provided for sperm flagella movement [5]. The effect of ATPe supplementation on mammalian sperm quality is still debated among laboratory groups i.e., in human [10,11], rat [5], and bovine [14] studies. In the studies on couples with infertility, sperm acrosomal exocytosis was not affected by ATPe, but the other sperm quality parameters, such as subjective motility, motility patterns, and fertilization rates, were influenced [10,11]. In contrast to bovine, ATPe supplementation stimulated sperm acrosomal exocytosis, the cause of sperm hyperactivation [14].

Because the domestic cat epididymal is susceptible to cryoinduced damage [8], it is a suitable model for other felid sperm function studies such as sperm metabolism, extracellular energy supplementation, and intracellular energy concentration. In addition, understanding the role of ATPe in cryopreserved sperm quality might help to improve sperm fertilizing ability in felids. Therefore, the objectives of this study were to (1) present the intracellular ATP level after postthaw epididymal cat sperm supplementation with ATPe and (2) evaluate effects of ATPe of different concentrations on postthaw epididymal cat sperm quality (proportion of motile sperm, progressive motility, membrane and acrosome integrity, and mitochondrial membrane potential) and fertilizing ability (homologous IVF).

2. Materials and methods

The study protocol was approved by the Academic and Research Department, Faculty of Veterinary Science, Chulalongkorn University, Bangkok, Thailand.

2.1. Experimental design

Eighteen adult male cats, subjected to routine castration at the Veterinary Public Health Division of the Bangkok Metropolitan Administration, Bangkok, Thailand, were included in this study. A pair of testis was kept in isotonic normal saline supplemented with 1% penicillin and streptomycin and transported to the laboratory within 3 hours. Epididymal sperm from two male cats were pooled (N = 9). Each pooled sperm sample was evaluated for sperm concentration, total number of sperm (adjusted to 18×10^6 sperm by discarding the excessive sperm from the pooled sample), morphology, subjective motility, membrane and acrosome integrity, and mitochondrial activity. Each sperm sample was then equally divided into three aliquots (6×10^6 sperm each), equilibrated by cold storage for 1 hour, and cryopreserved with the Tris–egg yolk extender. Before cryopreservation, epididymal sperm quality was repeatedly evaluated. After thawing, the sperm sample from the first straw was divided into three aliquots: control, supplemented with 1.0- or 2.5-mM ATPe and evaluated for sperm quality at 10 minutes, 1, 3, and 6 hours. The sperm sample from the second straw was divided into three aliquots: incubated without ATPe (control) and with ATPe supplementation (1.0 or 2.5 mM) for 10 minutes and evaluated for fertilizing ability by homologous IVF. Intracellular ATP concentration assessment was performed in the samples of ATPe concentration that provided the best sperm quality and fertilizing ability. The sample from the third straw was divided into three aliquots and one was discarded. The first and second sperm aliquots were divided into two groups: control and 2.5-mM ATPe supplementation. The intracellular ATP was evaluated by high-performance liquid chromatography (HPLC). The experimental design flow chart is shown in Figure 1.

2.2. Media

The chemical substances were purchased from Sigma Chemical Co., St. Louis, MO, USA, unless otherwise stated.

Sperm freezing extenders and thawing media were prepared according to the protocol described by Axnér et al. [15]. Briefly, epididymal sperm was diluted with the extenders composed of Tris–egg yolk extender I (EYT-I) and Tris–egg yolk extender II (EYT-II). The EYT-I was added to the sperm samples at room temperature. Tris–egg yolk extender II, which was added at 4 °C during an equilibration, had the same composition as EYT-I except that it contained 1% Equex STM Paste (Nova Chemical Sales, Scituate, Inc., MA, USA) and 7% glycerol. A Tris buffer having