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Differences in semen freezability and intracellular ATP content between the rooster (*Gallus gallus domesticus*) and the Barbary partridge (*Alectoris barbara*)

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Abstract

This study aimed to compare viability, ATP content, and DNA integrity of rooster (*Gallus gallus domesticus)* and Barbary partridge (*Alectoris barbara*) fresh and frozen spermatozoa in order to identify factors possibly related to differences in semen freezability. Ejaculates were obtained from March to May by the abdominal massage method from 3 adult roosters and 12 adult Barbary partridges. Semen was frozen with different cryoprotectants using Lake's diluents as a base medium: 1) glycerol 11%; 2) glycerol 11% and trehalose 70 mmol/L; 3) dimethylacetamide (DMA) 6%; 4) DMA 6% and trehalose 70 mmol/L. Both fresh and frozen semen showed a lower viability and higher intracellular ATP concentrations in the Barbary partridge compared with the rooster ($P < 0.05$). In the Barbary partridge, semen viability after thawing did not differ among the 4 media used, but glycerol showed positive effects in avoiding a significant loss of ATP after thawing, compared with DMA containing media ($P < 0.05$). On the other hand, in the rooster a higher viability was recorded when semen was frozen in glycerol containing media compared to DMA ($P < 0.0001$), while ATP values significantly decreased after thawing ($P < 0.05$) without showing any differences among the semen frozen in the 4 different media. DNA integrity, as evaluated by the comet assay, was assessed only in frozen semen. In the Barbary partridge, mean scored parameter did not differ significantly among semen frozen in the 4 different media. In the rooster DNA fragmentation was higher in DMA ctr medium compared with the other media and with values found in Barbary partridge semen frozen in the same medium ($P < 0.001$). In both species, the addition of trehalose did not show any positive effects on viability, ATP levels and DNA integrity after thawing.

In conclusion, species-related differences in semen features exist between the rooster and the Barbary partridge and the wide variation observed in ATP levels may account for differences in semen freezabililty between the two species.
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Keywords: Semen cryopreservation; Birds; DNA integrity; Trehalose

1. Introduction

The ability of sperm to survive cryopreservation and remain functional requires methodologies that are within the biophysical and biological limits defined by the cryo-

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biological characteristics of each species [1]. Significant differences exist among the commercial poultry species (turkey, broiler-type chicken, and layer-type chicken) in terms of the viability and functionality of sperm after cryopreservation. In particular, the fertility rates from frozen/thawed turkey semen consistently have been lower than cryopreserved chicken semen [2]. In the same way, semen cryopreservation in wild bird species lead to very wide differences in effectiveness according to species and breeding conditions [3–5].

Two biophysical traits of bird semen, i.e. resistance of spermatozoa to osmotic stress and membrane fluidity, have been reported to be possibly related to the differences between species and individual in the ability of spermatozoa to withstand the freezing-thawing process [4]. It follows that biochemical and molecular-biological comparisons of sperm among species may lead to identification of factors that influence the freezability of avian semen [6].

Energy metabolism is a key factor supporting sperm function. Sustaining sperm motility and active protein modifications such as phosphorylation could be the reason why sperm require exceptionally more ATP than other cells [7]. ATP is one of the basic components in a sperm cell and is used not only as an energy source but also for protein phosphorylation in cell signalling and as a cofactor regulating protein function [7]. In mammalian sperm, ATP production supports multiple cellular activities and biochemical events required for successful fertilization to occur, such as capacitation [8,9], acrosome reaction [10], and motility [7]. The functional integrity of mitochondria is believed to be important for sperm survival in the female genital tract or during assisted reproductive biotechnologies [11]. We have reported that individual differences exist in Griffon vulture spermatozoa ATP content and that a higher ATP concentration in fresh semen was followed by a longer survival *in vitro* after cryopreservation [12].

Among other sperm tests, evaluation of DNA integrity has been considered important in determining spermatozoa ability to withstand cryopreservation procedures. After cryopreservation, in fact, spermatozoa are particularly susceptible to DNA damage since freezing and thawing procedures lead to significant reduction in the level of spermatozoa antioxidant [13]. We reported that Griffon vultures frozen/thawed spermatozoa do not appear to be particularly susceptible to DNA fragmentation during cryopreservation [12]. Unfortunately, even if the assessment of DNA integrity is of high value in determining frozen/thawed semen quality, extensive studies on avian semen DNA integrity after cryopreservation are lacking.

The aim of the current study was to compare viability, ATP content, and DNA integrity of rooster (*Gallus gallus domesticus*) and Barbary partridge (*Alectoris barbara*) fresh and frozen spermatozoa in order to identify factors possibly related to differences in semen freezability. In addition, different permeable cryoprotectants [glycerol (Gly) and dimethylacetamide (DMA)] and an additive (trehalose; trh) were tested in order to identify the most suitable protocol for Barbary partridge semen cryopreservation. The Barbary partridge is listed as Species of European Conservation Concern (SPEC) 3 in the SPEC list, but the actual status of *A. barbara* populations and their main threats are poorly known [14]. Nowadays, the only non-African regions where this species can be found are the Gibraltar peninsula, Sardinia and the Canary islands [15]. Signs of genetic impoverishment have been found in the Sardinian wild population, possibly due to local inbreeding or to extensive restocking with inbred captive-reared birds [16]. Conservation and breeding programs would therefore benefit from the creation of a sperm cryobank aimed at preserving the genetic diversity of the population kept in captivity for breeding purpose [17,18].

2. Materials and methods

2.1. Chemicals

All reagents and media were from Sigma Co. (St. Louis, MO, USA) unless otherwise specified.

2.2. Birds

All experimental procedures were carried out at the experimental facilities of the Department of Animal Biology at the University of Sassari, Italy (latitude 40 °43′ N). These facilities meet the requirements of the European Union for Scientific Procedure Establishments. This study followed ethical guidelines for care and use of agricultural animals for research (EC Directive 86/609/EEC for animal experiments). Only reproductively active males previously known to produce semen and proven to be fertile were chosen for this study. All birds were housed in outdoor individual pens under natural photoperiod and kept in visual contact with female conspecifics. Feeding schedules and bird handling were kept constant throughout the study. For this study 3 adult roosters and 12 adult Barbary partridges were used.

Semen collection attempts were made twice a week from March to May during two consecutive years (2007

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