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Semen evaluation in four autochthonous wild raptor species using computer-aided sperm analyzer

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ABSTRACT

At least 10 percent of the approximately 300 species of the order Falconiformes are listed as being globally threatened. The present work describes the seminal characteristics of three diurnal and one nocturnal raptor species. Semen was collected from clinically healthy *Accipiter nisus* (n = 1), *Falco subbuteo* (n = 6), and *Falco tinnunculus* (n = 5) adult males that were housed at the 'Centro Animali Non Convenzionali' of the Department of Veterinary Sciences of the University of Turin. The semen was collected after a period of recovery and before their release as well as from seven *Bubo bubo* males bred in captivity as part of a raptor conservation project. All the potential semen donors were trained in semen collection during the breeding season via a ritualized procedure. Ejaculation was achieved using a massaging technique. Each sample was evaluated for volume, degree of contamination, and spermatozoa concentration. The semen motility and kinetic parameters were assessed on diluted semen (modified tyroides albumin lactate pyruvate, pH 7.5, temperature 37.5 °C) using a computer-aided sperm analyzer. Semen collection was successful in all the diurnal species and in five *B bubo* individuals. The sperm motility and sperm kinetic parameters were very variable both among and within species. In contrast with previous studies that involved raptors bred in captivity and imprinted on humans, we worked with wild birds and attempted to overcome the problem of poor semen quality, which is strongly influenced by stress, by adopting a ritualized procedure that has never been reported for semen collection purposes.

1. Introduction

Knowledge of the seminal characteristics of a species is crucial for understanding their reproductive biology and particularly for planning captive breeding programs that adopt AI. AI can be used in avian conservation programs to assist in creating viable, self-sustaining populations [1]. At least 10% of the approximately 300 species of the order Falconiformes are listed as being globally threatened [2]. Apart from the excellent results obtained with selected peregrine falcons [3,4] and California condors [5], captive breeding, especially that of endangered eagles and hawks, is far from successful [6]. This is partly because of the inadequate knowledge of the normal reproductive parameters of various species but it is also because of the likely consequence of captivity stress [7]. Additional problems with wild raptors include the unavailability of founders, inbreeding depression, female-male incompatibility, asynchrony, inability to naturally copulate, poor semen quality and urine contamination when birds are brought into an *ex situ* environment, sperm transport inefficiency, and diseases [6]. Common species can be used as a model for endangered ones, both for semen collection, processing, and preservation as well as for captive breeding programs [8]. Although many wild raptor species are not endangered, very little is known about their seminal characteristics

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[9,10]. The common kestrel (*Falco tinnunculus*) and Eurasian sparrowhawk (*Accipiter nisus*) are two raptor species that are considered to be residents in Italy. Only Northern Europe populations migrate south for the winter, whereas their southern counterparts, at most and in rare cases, show limited dispersive movements [2,11]. These two species represent the most common birds of prey in Europe. Conversely, the Eurasian hobby (*Falco subbuteo*) is a long-distance migrant that winters in Africa and Asia. This species is largely present in Italy, but it is a more vulnerable species that mainly eats insects, swifts, and house martins. The Eurasian eagle-owl (*Bubo bubo*) is a species of eagle-owl that resides in much of Eurasia; besides being one of the largest living species of owl, it is also one of the more widely distributed. With a total range in Europe and Asia of approximately 32 million square kilometers and a total population estimated to be between 250 thousand and 2.5 million individuals, the 'International Union for Conservation of Nature' lists its conservation status as being of "least concern" [12]. Various studies have investigated the biology or breeding behavior of this species and report reproductive programs in captivity [13,14], but none has addressed semen collection and evaluation.

The present study aims to improve the knowledge of the seminal characteristics of four common raptor species using CASA, which is a standardized and objective evaluation method.

2. Materials and methods

The 'Centro Animali Non Convenzionali' (C.A.N.C.) of the Department of Veterinary Sciences of the University of Turin treats injured wild animals with the goal of releasing the ones that recover. C.A.N.C. has a project to build a wild avian species semen bank for conservation purposes, and the present study is part of that project. In the breeding season (April–June) of three consecutive years (2013–2015), attempts at semen collection were made in all the clinically healthy adult males of *A nisus* (n = 1), *F subbuteo* (n = 6), and *F tinnunculus* (n = 5) that arrived at the 'Centro'. At the same time and in the full breeding season (February–May) of 2014, seven adult Eurasian eagle-owl males (*B bubo*) between 7 and 15 year old were included in the study. All the owls were housed in outdoor pens, coupled with a female, and bred in captivity, in agreement with C.A.N.C. raptor recovery and conservation projects. Every raptor species, both diurnal and nocturnal, was fed a diet consisting of rabbits, quails, rats, day-old chicks, mealworms, and locusts (only *F subbuteo*) in varying percentages, depending on the species.

All the males were both macroscopically and endoscopically evaluated for the confirmation of good clinical conditions and for the assessment of gonadal status and functionality. The potential semen donors were trained in semen collection twice weekly, beginning with a ritualized procedure consisting of a fixed hour of performance, the affixation of a falconry hood immediately after capture, precise positioning, bandaging of the talons with cohesive bandaging tape (Vetrap), and a series of simulated semen collection manipulations. The procedure was always consolidated with positive reinforcement consisting of the daily meal at the conclusion of the process. In the diurnal raptor species, the semen was collected in the early morning (between 8:00 and 10:00 AM.) and in the early afternoon (between 02:00 and 04:00 PM.) in the Eurasian eagle-owl. For semen collection, each bird was physically restrained by an operator using a soft towel to contain the front half of the bird's body to avoid struggling and stress and to ensure safety. Ejaculation was achieved using a modified massaging technique [15] with the thumb and index or middle finger on the dorsal aspect of the abdomen toward the cloaca, followed by gentle rhythmic squeezing at the base of the cloaca with the same finger of the other hand. The ejaculate was collected in graduated microcapillary tubes (Microcaps; Drummond Science Company Broomall, PA, USA) and directly evaluated for color and volume. Immediately after collection, the semen was empirically diluted, from a minimum of 1:2 (*B bubo*) to a maximum of 1:50 (*Falco* sp.), in modified tyroides albumin lactate pyruvate (100-mM sodium chloride; 3.1-mM potassium chloride; 25-mM sodium carbonate; 0.3-mM sodium dihydrogen phosphate; 10-mM HEPES; 2-mM calcium chloride; 0.4-mM magnesium chloride, and 1mg/mL sodium pyruvate). All the components were from Sigma–Aldrich (St. Louis, MO, USA), calibrated at pH 7.5 and maintained at 37.5 °C. The time from semen collection to analysis was within 5 minutes. The degree of contamination of the diluted ejaculates was visually classified from 1 to 5, and the type of the contaminants was recorded (urates, erythrocytes, and feces). When the contamination degree was greater than 4, the samples were discarded. The sperm concentration was determined using a Makler chamber after a standard 1:100 dilution of 10 μL of the extended sample with a solution of distilled water and 4% formaldehyde. Semen motility and the motility parameters of 10 μL of the extended semen placed in a preheated Makler chamber (37.5 °C) were evaluated using a Computer Aided Sperm Analyzer (CASA; CEROS, Hamilton Thorne Research Inc., version 14 Build 008, IMV Technologies, France). The evaluated parameters were total motility (TM %), progressive motility (PM %), average path velocity (VAP μm/s), straight line velocity (VSL μm/s), curvilinear line velocity (VCL μm/s), amplitude of later head displacement (ALH μm), beat cross frequency (BCF Hz), straightness of the track (STR %), and the linearity of the track (LIN %). The settings of the instrument were as follows: 60 frames per second (Hz), 30 frames per field, minimum contrast = 15, minimum cell size = 10; and static cells were considered when average path velocity less than 10.0 μ/s and straight line velocity less than 13.0 μ/s. These parameters were chosen after the different trials with raptor species semen (data not shown).

3. Results

The semen collection was successful in 1/1 individual birds of *A nisus*, 6/6 *F subbuteo*, 5/5 *F tinnunculus*, and 5/7 *B bubo*. The number of attempts at semen collection in the different species is reported in Table 1. *A nisus* and *F tinnunculus* required an average of 3 weeks of training, whereas *F subbuteo* had to be trained for a longer period of 4 to 5 weeks. Although an analyzable sample could be