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Seasonal and genera-specific variations in semen availability and semen characteristics in large parrots

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ABSTRACT

In large parrots electro-stimulation is suitable for collecting semen, and therefore, to facilitate semen examination and artificial insemination. Previous studies have detected differences in the semen collection success rate and semen parameters between psittacine genera. It remained unclear whether these differences were genera-related, seasonal variations or depend on the males' relationship status. To answer these questions, semen collection and spermatological analysis were performed for four psittacine groups (macaws, amazons, eclectus parrots and cockatoos) over 13 months. In one breeding facility, semen collection was attempted in 82 males using electro-stimulation twice monthly. A complete spermatological evaluation was performed on 435 semen samples. Volume, color, consistency, contamination and pH of semen, as well as motility, progressive motility, sperm concentration, total sperm count, viability, and morphology of spermatozoa were evaluated. Seasonality affected the collection success rate in macaws and amazons. Thereby, in amazons a distinct peak was observed several days before and around oviposition, whereas eclectus parrots and cockatoos produced semen all year round. The average sperm concentration was highest in eclectus parrots (2.7×10^6 sperm/μl) and lowest in macaws (35.6×10^3 sperm/μl). The differences in the semen collection success rate and semen parameters seem to coincide with the bird's breeding biology. The collected data allows a prognostic estimation when semen collection seems favorable, and may be taken as orientation values for semen analysis in these species.

1. Introduction

The destruction of natural habitat as well as nest poaching and illegal trade has put many psittacine species on the Red List of threatened species of the International Union for Conservation of Nature [1–3]. In addition to the efforts for conservation of the natural distribution areas, ex-situ actions such as captive breeding programs are important tools for the conservation of endangered parrots [4,5]. Unfortunately, a common problem in captive breeding programs of highly endangered psittacines is the high proportion of infertile eggs, which may be attributed to disharmony of forced-paired individuals and male infertility [6]. Semen analysis may help to identify and replace presumably subfertile or infertile individuals in a breeding program and to classify fertile males according to sperm quality in order to identify suitable males as semen donors for assisted reproduction techniques [6]. Semen of good quality is essential for successful artificial insemination, which may be a useful tool to overcome reproductive failure, aiming for a stable captive population and reintroduction programs [7].

Semen collection and artificial insemination have been established for domestic fowl [8] and birds of prey [9] for decades. Here,

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data related to semen quality is well known [10,11]. Assisted reproduction techniques are also established for some smaller psittacines and first spermatological data is available [12–16]. Until recently, in larger parrots, semen collection has only been reported sporadically and with moderate success [17,18]. By using a new technique based on electro-stimulation, it was possible to collect semen from more than 100 psittacine taxa [6]. In the mentioned study, differences in the success rate of semen collection and in semen parameters have been detected between the different psittacine genera. It has been shown that the average sperm concentration was much higher in eclectus parrots (3.8×10^6 sperm/μl) than in other psittacine genera such as macaws (7.0×10^4 sperm/μl) [6]. However, it remained unclear if these differences were related to the selection of males and their relationship status (single versus paired) or to seasonal aspects, as the study was performed only for three months in the spring.

Reliable spermatological reference values of large parrots are still lacking. Moreover, it is unknown whether and how semen quality varies during the year. In rosy-faced lovebirds (*Agapornis roseicollis*), ejaculate volume and sperm concentration were found to be significantly lower outside of breeding season [16]; nevertheless, similar studies are lacking for larger parrots. Given that most large psittacines such as amazons, macaws and cockatoos have a relatively short annual period of sexual activity [7], the season may affect semen parameters and, therefore, influence the success of artificial insemination.

The objective of this study was firstly to evaluate the influence of the season on the success rate of semen collection and on semen parameters as well as to create spermatological orientation values for large parrots. These results may be of importance to develop cryopreservation techniques for parrots in order to establish semen banks for endangered species. Secondly, semen collection success rates and semen quality were compared between paired males and singly housed males. Thirdly, semen parameters of amazons were studied particularly around the time of oviposition by the female partner.

2. Material and methods

In total, 82 males of 33 different psittacine species or subspecies, consisting of 37 amazons (*Amazona* spp.), 21 cockatoos (*Cacatua* spp. n = 18, *Calyptorhynchus* sp. n = 3), 14 macaws (*Ara* spp. n = 11, *Diopsittaca* sp. n = 1, *Primolius* spp. n = 2) and 10 eclectus parrots (*Eclectus* spp.) from a single breeding facility in Tenerife (Spain) were included in the study (Table 1). The birds were housed in outdoor aviaries paired with a female of the same species (n = 60), in a male group (n = 3) or as single males (n = 19). Paired males and males without a female partner were evaluated separately. The group of paired males included proven fertile males, which produced offspring in the past and males from couples having a history of infertile clutches. All macaws and eclectus parrots were paired with female conspecifics (Table 1). Animals were fed fruits, vegetables, boiled pulses and vitamin mineral supplement in the morning and a species-specific seed-pellet mixture in the afternoon. Fresh drinking water was available ad libitum. No artificial modification of temperature, humidity or light intensity was performed. The clinical health of the birds was checked daily and the absence of viral infections and endoparasites was checked twice annually.

In every individual male semen, collection was attempted twice monthly with an interval of two weeks. Semen collection was performed in the morning between 8:00 and 10:00 a.m. and in the afternoon between 2:00 and 4:00 p.m. Animals were not fasted before semen collection. The collection period lasted 13 months from June 2012 to June 2013. Immediately prior to the procedure, the animals were netted from the aviary and manually restrained by one helper. Semen collection was performed by one examiner via electro-stimulation as previously described by Lierz et al. (2013) and approved by the Regierungspräsidium (Regional Council) Giessen, Germany; with the permission number V54-19C20-15(1) GII8/9 Nr. 77/2009. Three different sizes of a bipolar probe (length: 2.5, 3.5, or 5 cm; diameter: 3, 4 or 5 mm) were used for the different species. The probe size, the electrical current (1–6 V) and the number of electric impulses (2–6 intervals, each lasting 1–2 s with a 1 s free-interval) were individually adapted to each bird. Starting with a low voltage (1 V), the series was repeated a maximum of three times with increasing current (max. 6 V) until soft contraction of the cloacal musculature and the muscles of the tail was observed. Semen was collected from the cloaca using scaled glass capillaries (Wiretrol II, 1–5 μl; Drummond Scientific Company, Broomall, PA, USA). If semen emission could not be achieved within five minutes or a maximum of three repetitions of six electrical impulses, attempts were recorded as unsuccessful.

When semen was obtained, the glass capillary with the semen sample was kept light-protected in a plastic box at ambient temperature until analyses were performed, 10–15 min after sampling.

Initially, samples were analyzed directly in the capillary for volume, color (categories: colorless, grayish, whitish, greenish, brownish, yellowish, reddish), consistency (categories: watery, whey-like, milky, creamy), and contaminations (urates, feces, blood) as well as pH by using a pH indicator paper (Special indicator pH 6,4–8,0; Macherey-Nagel, Dueren, Germany) as previously described for psittacines [19]. Sperm concentration was estimated by microscopy (100× magnification, Hund Wilozyt V365; Helmut Hund GmbH, Wetzlar, Germany) directly in the scaled glass capillary and graded from + to +++ [14]. According to the obtained volume (minimum of 2 μl) and estimated sperm concentration, samples were diluted with a modified Lake diluent [20] 5-, 10- or 50 fold, respectively, and forwarded to a detailed semen analysis, including sperm motility, progressive motility of spermatozoa, sperm viability, sperm concentration, total sperm count and sperm morphology.

Sperm motility was determined on a pre-warmed (37 °C) slide estimating the average percentage of motile and progressively motile spermatozoa in five fields of view (400× magnification, Hund Wilozyt V365; Helmut Hund GmbH, Wetzlar, Germany) according to previously described procedures for psittacines [15]. Sperm concentration was assessed by using a Neubauer counting chamber after a 1:10 dilution of the extended sample with distilled water. Total sperm count (TSC) of the ejaculate was generated by multiplying sperm concentration with semen volume [21]. Eosin B (2%) staining was used for evaluating sperm viability according to previous studies [15]. In eosin B stained semen smears, living sperm (white) were distinguished from dead sperm (colored red), and the number of viable sperm was determined as the percentage of total spermatozoa. For preservation, these stained smears were covered with a mounting medium (Entellan New, 107961; Merck KGaA, Darmstadt, Germany) and cover slips before sperm morphology was analyzed [19].

If available, five semen smears from each month and of each group were selected randomly out of all samples for sperm morphology analysis. In total, 178 smears (amazons: 39, cockatoos: 60, macaws: 19, eclectus parrots: 60) were evaluated. In each smear, 200 spermatozoa were categorized as normal or malformed by following the standardized evaluation of sperm morphology [22]. The following categories were used for malformed sperm cells: head abnormalities, midpiece abnormalities, and tail abnormalities.

To determine significant differences in semen volume, sperm concentration and total sperm count between the four groups,