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Immature and mature sperm morphometry in fresh and frozen-thawed falcon ejaculates

S. Villaverde-Morcillo [a], A.J. Soler [b], M.C. Esteso [c], C. Castaño [c], A. Miñano-Berna [d], F. Gonzalez [e], J. Santiago-Moreno [c, *]

[a] Veterinary Clinical and Research Services, Fieb Foundation, Madrid, Spain
[b] SaBio IREC (CSIC-UCLM-JCCM), Campus Universitario, Albacete, Spain
[c] Department of Animal Reproduction, INIA, Madrid, Spain
[d] CRFS El Valle, Consejería de Agricultura y Agua, Región de Murcia, Murcia, Spain
[e] Wildlife Rehabilitation Hospital GREFA, Majadahonda, Madrid, Spain

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ABSTRACT

Sperm morphometry is one characteristic which may be useful in prediction of fertility and sperm freezability in a species. Knowledge of the sperm characteristics of the ejaculate and the morphometric descriptors is necessary to effectively develop sperm cryopreservation. The aim of the current study was to provide a general description of the sperm from two falcon species (Peregrine falcon *Falco peregrinus peregrinus/brookei* and Gyrfalcon *Falco rusticolus*) including immature sperm, sperm head morphometric descriptors, and the existence of mature sperm subpopulations.

Semen samples were collected by massage and voluntary false copulation and diluted with Lake and Ravie medium. Smears were prepared of the diluted samples, stained with Hemacolor®, and subjected to: 1) morphological analysis (bright field optical microscopy), and 2) computerised morphometric analysis; each sperm head was measured for length, width, area and perimeter. In addition, in the Gyrfalcon, pooled semen was frozen in pellets using DMA as a cryoprotectant and the analyses repeated after thawing. The mean percentage of immature sperm (spermatocytes and spermatids) was similarly high in all species/subspecies: Brookei Peregrine falcon (*F. p. brookei*) 55.5%, European Peregrine falcon (*F. p.* peregrinus) 65.5% and Gyrfalcon 64.7%. Clustering analyses identified four subpopulations of mature spermatozoa with different morphometric characteristics ($P < 0.001$). The relative proportions of these subpopulations were similar in all three species. The mean values recorded for the morphometric variables of the four subpopulations were, however, lower ($P < 0.001$) in the thawed Gyrfalcon samples than in fresh samples. The results support the idea of pleiomorphy as a characteristic of raptor mature sperm. This finding, plus that of the existence of four sperm subpopulations with different morphometric characteristics, may be important in the future development of cryopreservation protocols for falcon sperm.

1. Introduction

The family Falconidae is represented by 66 species around the world, of which 18 now appear on the IUCN Red List as facing some threat of extinction. Habitat fragmentation [1], pollution [2] and climate change [3] remain constant threats. While habitat protection, species protection and threat management strategies all have their place in the conservation of these falcons, captive breeding and the banking of genetic resources appear as increasingly important options [4–7]. Establishing self-sustaining captive populations of different wildlife species is a major conservation goal [8] although it requires large numbers of animals to be maintained [9–11], and the space available in zoological gardens, animal parks and research centres is limited. In contrast, the cryopreservation of germplasm offers a means of preserving genetic variation without large space requirements [4,12,13]. Further, the cryopreservation of germplasm would allow the controlled "breeding" of individuals of genetic interest [12,14,15] even after their death [16].

Artificial reproduction techniques have been widely used in

* Corresponding author.
E-mail address: moreno@inia.es (J. Santiago-Moreno).

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human and domestic animal species and are now being transferred to wild species. Artificial insemination using fresh or frozen semen is the method most commonly employed [17–19]. For many species, however, basic spermatological and cryobiological studies still need to be performed if the best results are to be achieved [20–23].

In some species, such as pheasants [24], passerines [25], goats [26], stallions [27], bulls [28,29], red deer [30], and boar [31,32], the fertilizing capacity of spermatozoa and even their response to certain assisted reproductive techniques, such as the cryoresistance to freezing-thawing process, may be strongly influenced by their morphological and morphometric characteristics. The same may be true for raptor sperm. In addition, the morphometric alterations caused by freezing might affect the quality of thawed raptor sperm as reported in other species [30,31].

The shape and size of the sperm are important indicators of quality and freezing capacity of a given sample [32]. The existence of sperm head morphometric-subpopulations has been identified in some mammalian species [34,35]. Semen samples more resistant to cryopreservation have a higher percentage of sperm with small head area [32]. Thus, descriptive analysis of sperm morphometry and of the existence of sperm sub-populations is useful to improve the probability of effectively developing sperm cryopreservation. With a view to understanding more about the optimum conditions required to improve cryopreservation of falcon sperm, the aims of the present work were to determine the proportions of mature and immature sperm cells (morphological analysis) and to identify mature sperm subpopulations according to sperm head morphometric descriptors (morphometric analysis) in ejaculates from Peregrine falcons (*Falco peregrinus*) and Gyrfalcons (*Falco rusticolus*). In addition, the influence of freezing-thawing on sperm head morphological and morphometric variables was studied using pooled Gyrfalcon semen as a model.

2. Materials and methods

2.1. Experimental birds

Sperm was collected from three Brookei Peregrine falcons (*F. p. brookei*), two European Peregrine falcons (*F. p. peregrinus*) (the property of Sevilla Falcons S.L. Seville, Spain and the *Centro de Halcones de Madrid*, Madrid, Spain), and 23 Gyrfalcons (housed at the Roc Falcon S.L. Breeding Centre in Lleida, Spain). All birds were mature (between 4 and 8 years), considered fertile and healthy. They were housed in open facilities under natural photoperiod with *ad libitum* access to water. The birds were housed individually and some of them had visual contact with females. The diet were mainly composed by combining one day old chicks and quails; a plus of vitamins Nekton-E was added to the food following the recommendations of the manufacturer. Handling procedures were performed in accordance with the Spanish Policy for Animal Protection RD53/2013, which conforms to European Union Directive 86/609 regarding the protection of animals used in scientific experiments. The FIEB Ethics Committee approved the entire study (Animal Care Committee approval number 20150202/0001).

2.2. Semen collection

Thirty semen samples were collected from the three Brookei Peregrine falcons (10 samples per bird), 20 from the two European Peregrine falcons (10 samples per bird) and 34 from the 23 Gyrfalcons (one - three samples per bird). Semen samples were collected using either abdominal massage [36] as adapted to these species [37] or voluntary false copulation (imprinted birds only: 2 Brookei, 1 European and 3 Gyrfalcons) [38] in the middle of the breeding seasons (March and April), discarding early and late sperm samples. All samples were recovered by capillarity using glass capillaries (75 μL Globe Scientific Inc, Paramus, New Jersey, USA), and then transferred into 1.5 mL Eppendorf® microcentrifuge tubes (Eppendorf Ibérica SLU, San Sebastián de los Reyes, Madrid, Spain). Semen was usually collected every 48 h in Peregrine falcons and Gyrfalcons, but this interval could vary, shorter or longer, if necessary according to the demand of artificial inseminations in the Breeding Centres. The volume of each sample was immediately recorded before diluting. It was then diluted 1:1 (v:v) with Lake-Ravie medium [39] at ambient temperature. This extender was prepared using reagent-grade chemicals purchased from Panreac Quimica S.A. (Barcelona, Spain) and Sigma Chemical Co (St. Louis, Missouri, USA).

2.3. Sperm analysis

Sperm morphology and head morphometry were examined as previously described [40] using a Motic BA 210 optical microscope (Motic Spain, S.L.U. 08349 Cabrera de Mar, Barcelona, Spain) with a 100× oil immersion objective lens (bright field) and Motic Image Advanced v.3.0 software (Motic Spain, S.L.U. 08349 Cabrera de Mar, Barcelona, Spain). Smears were prepared by spreading 5 μl drops of diluted semen samples onto glass slides and allowing them to air-dry at room temperature for about 2 h. Smears were then stained with Hemacolor® (Merck KGaA, 64271 Darmstadt, Germany) using Coplin jars: the smears were fixed by dipping the smears 2 min in fixative solution and stained with corresponding kit's acid and basic stains by dipping the smears for 2 min in each. Finally, smears were air dried (about 2–3 h), and the slides were then sealed with

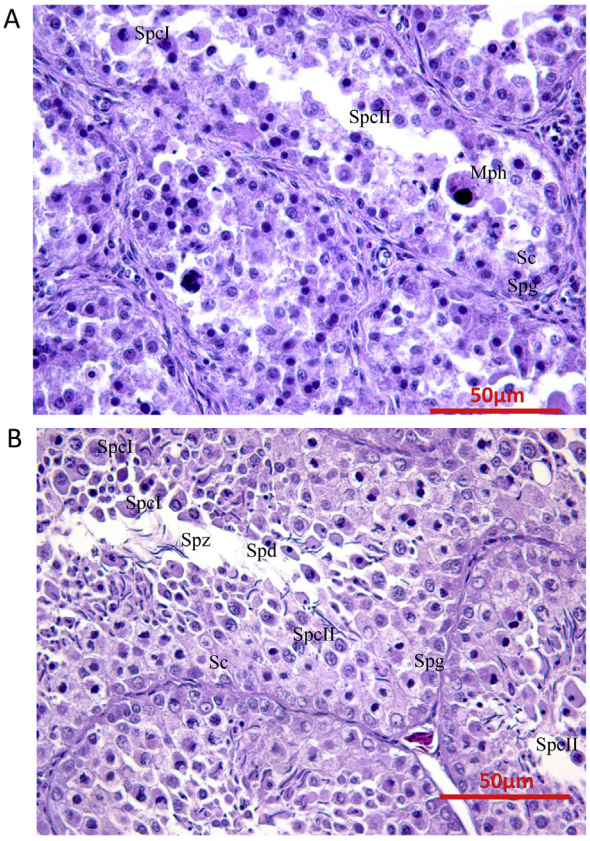

Fig. 1. Cross-sections of seminiferous tubules from a sexually inactive (A) and a sexually active (B) Peregrine falcon, respectively. Spermatogonia (Spg); Primary spermatocyte (SpcI); Secondary spermatocyte (SpcII), Spermatid (Spd), spermatozoa (Spz); Sertoli cell (Sc); Macrophage (Mph) (Haematoxylin Eosin, ×400).