



Comparison of celioscopy and histological examinations to assess male gonadal health and functionality in adults and immature wild raptors



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ABSTRACT

Celioscopy is routinely used in birds for sex determination and diagnostic purposes. Aim of this work was to validate celioscopy for the assessment of male gonads functionality in wild raptors, comparing the results of direct observation with morphometrical and histological characteristics. The work was done at the 'Centro Animali Non Convenzionali' of the University of Turin, Italy, on 31 endoscopically evaluated raptors that died or were euthanized. Through celioscopic observation, the birds were classified in adults or immatures and maturity categories were defined according to the adrenal-gonad size ratio and to the degree of blood filling of testicular vessels. The gonads were removed immediately after death/euthanasia and measured. Albuginea tunic thickness, diameter of seminiferous tubules, number of meiosis figures, tubular development degree, tubular degeneration degree and germinal cells production degree were evaluated. Testicular size tended to increase from immature to adult birds and from 'out of' to 'in' breeding season; albuginea tunic thickness tended to be higher out of the reproductive season while diameter of the seminiferous tubules, germinative epithelium thickness and number of meiosis figures were higher in the breeding season. In season adults generally showed higher values in tubular development and germinal cells production, and lower degrees of tubular cells degeneration and fibrosis. From the interpretation of all the morphometrical and histological aspects, a general reproductive degree of activity was given to the birds and compared with celioscopic results. A perfect concordance was found in 23 out of 31 cases and a good concordance in six ones; histology could describe obviously better sub-clinical conditions undetectable at direct observation. These preliminary results suggest that celioscopy could be a useful tool to assess male gonads functionality in wild raptors, with the future goal to select the better potential semen donors.

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1. Introduction

At least 10% of the approximately 300 species of the Order *Falconiformes* are listed as being globally threatened [1]. Apart from the excellent results obtained with some species as Peregrine falcon [2] or California condor [3], captive breeding, especially that of endangered eagles and hawks, is far from successful [4]. This is partly due to the inadequate knowledge of their physiological reproductive aspects, but it is also due to the likely consequence of

captivity stress on gonadal activity [5,6]. Selection of semen donors represents the first step of artificial insemination, a technique that has become an important component of recovery and conservation programs for many raptors species, although it unfortunately represents only one of the many difficult aspects of captive breeding [5]. Common species can be used as models for endangered ones but the knowledge of reproductive physiology and anatomy is very limited also in many not endangered wild raptor species. Celioscopy can be routinely used for sex determination in those raptor species where no evident sex dimorphism exists but also for diagnostic purposes, through the direct observation of viscera and the collection of microbiological or biopsy specimens [7–9].

Studies on the anatomy and histology of wild raptors gonads are

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very limited and little is known about their sexual maturation, their gonadal cycle and spermatogenic activity, both in juveniles and adult subjects [10]. Usually, at Italian latitude, wild birds are seasonal breeders and their reproductive organs change in a cyclical manner. During the breeding season, the testes are maximally developed and hormonally stimulated, for the purpose to guarantee the highest numerability and fertility of gametes [11]. Once the breeding season has finished, there is an involution phase that leads to total inactivity of the male reproductive tract and represents a period of rest in preparation for the next breeding season [11]. Aire [11] identifies four testicular phases: *Progressive*, *Culmination*, *Regression* and *Resting*. During the *Regression* phase involutive or regressive changes occur throughout the testis, from the testicular capsule to the core of the seminiferous epithelium. The testicular capsule will be gradually replaced by a new capsule, generated by fibroblast proliferation [12]. The seminiferous tubules are physiologically atrophic and contain only spermatogonia and few primary spermatocytes [11]. The removing of all degenerating cells is carried out by macrophages, transiently invading the seminiferous tubules from the interstitial blood-vessels, through the lamina propria and basal lamina of the seminiferous tubules, and across the germinal epithelium into the lumen of the tubules [13].

The testicular framework is then ready for regeneration following hormonal and environmental external stimuli [11]. In the *culmination phase*, on the contrary, a male bird is morphologically and functionally ready to undertake reproduction, with spermatogenesis at the maximum peak of its efficiency [11].

The aim of this work was to validate the celioscopic examination of wild raptors male gonads through the comparison of direct observation results with morphometrical and histological characteristics, with the future goal to use celioscopy for selecting the better potential semen donors.

2. Materials and methods

2.1. Experimental birds

The work was done at the 'Centro Animali Non Convenzionali' (C.A.N.C.) of the Department of Veterinary Sciences of the University of Turin, Italy, a centre that takes care of injured wild animals, with the goal to return them into the wild, and is also involved in projects concerning the protection and conservation of some endangered species.

At C.A.N.C., birds of prey and owl species are routinely submitted to clinical celioscopy, as a routine procedure for sex determination and for diagnostic purposes. The immature birds and those with curable injuries, after first aid and stabilization, are subjected to celioscopy during hospitalization and rehabilitation time; when clinical conditions are untreatable, euthanasia is performed after anaesthesia. All the biological material used to perform the present study was collected for diagnostic purposes using standard clinical procedures. The study was performed in accordance with the guidelines for the care and use of animals of the Department of Veterinary Science of the University of Turin and with the consent of "Città Metropolitana di Torino" (local wildlife management district).

From January 2012 to July 2016, 206 birds of prey (42.7% of the total number of raptors admitted) and 156 owl species (44.9% of the total number admitted) were evaluated by celioscopy: 31 males died or were euthanized and their gonads were collected. They were 28 diurnal species and 3 nocturnal ones. The birds of prey belonged to different Genera (*Accipiter*, *Buteo*, *Falco* and *Pernis*) and were: *A. nisus* (N = 5), *A. gentilis* (N = 1) *B. buteo* (N = 13), *F. peregrinus* (N = 1), *F. tinnunculus* (N = 7) and *P. apivorus* (N = 1). The nocturnal birds were 3, all belonging to the same species of

owls, *Athene noctua*. The out-of-reproductive season or in-reproductive-season condition was registered.

2.2. Celioscopy procedure

All birds were anesthetized by isoflurane in 100% oxygen, in right lateral recumbence. When the clinical conditions of the birds were untreatable, euthanasia was done during the celioscopic evaluation. The entry site for celioscopy was caudal to the femur, on the left side of the coelom. The choice to operate on the left side depends on the fact that only the left ovary and oviduct are developed in the female birds of almost all the species that are the object of this study. In the males, both testes were evaluated through the same entry site, slightly rotating the bird during the celioscopic procedure. The endoscopic evaluation was performed with a 2.7–30° offset rigid endoscope (Karl Storz GmbH, Tuttlingen, Germany), while illumination was provided by a 250-W cold light source and fiberoptic cable (69495 NE, Karl Storz GmbH, Tuttlingen, Germany). After removing few feathers to expose the flank, a 3 mm skin incision, followed by gentle blunt dissection with small curve mosquito forceps, permits the entry of the endoscope between the last rib and the pubic bone, ventrally to the *flexor cruris medialis* muscle. The cranial extension of the limb maximizes the exposure of the caudal flank region and caudal internal visceral area of view, making also possible the evaluation of heart, caudal portions of lungs, liver, gastrointestinal tract, spleen, urogenital tract, cranial thoracic, caudal thoracic and abdominal air sacs. The exam led to the identification of sex and maturity degree of the birds, features that were not evident through any morphological trait.

Trough endoscopic evaluation of the gonads, the raptors were first classified as *adults* or *immature* birds. A further classification of *adults* into *sexually inactive*, *normal* and *sexually active* and of *immature birds* into *immatures* or *sub-adults* was defined observing the adrenal-gonadal size ratio and the degree of blood filling of the superficial vessels of the testicle capsule (Fig. 1). The length of each testicle was estimated in relation to the length of the left adrenal gland (the right adrenal gland being scarcely visible through a left entry site) [14]. The *sexually inactive adult* has an adrenal-gonad size ratio of 1:2 and a weak degree of blood filling; the *normal adult* has a size ratio ranging from 1:2 to 1:5, with a moderate degree of blood filling; the *sexually-active adult* has a size ratio higher than 1:5 and a high degree of blood filling. The *sub-adult* is different from the *immature* subject because it has intermediate characteristics between an immature subject and a sexually inactive adult, with an adrenal-gonad size ratio greater than 1:1 and an occasional presence of weak blood filling of the capsular superficial vessels.

2.3. Morphological and histological exam of the gonads

The gonads were removed immediately after death/euthanasia, in order to prevent any autolytic phenomenon; their longitudinal and transversal diameters were measured with a caliber and then they were fixed in Bouin's solution for 24 h, followed by 50% ethanol. Each testicle was then cut into two equal parts along the median sagittal plan and each half was included in paraffin. Three two-µm thick serial sections were cut from both the middle halves and stained with hematoxylin–eosin for histological evaluation. Particular attention was given to morphometric variations, to the germinative portion and the reproductive stages of the gonadal cells, but also to possible alterations as fibrosis, degenerative conditions of the germinative epithelium and inflammatory processes. The following morphological and anatomical aspects were taken into account and measured: thickness of the albuginea tunic; diameter of seminiferous tubules; thickness of the germinative

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