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

Andrea Dogliero a, *, Giacomo Rossib, Mitzy Mauthe von Degerfelda, Giuseppe Quarantaa, Ada Rotaa

a Dipartimento di Scienze Veterinarie, University of Turin, Italy
b School of Biosciences and Veterinary Medicine, University of Camerino, Italy

Article info
Article history:
Received 2 February 2017
Received in revised form 20 July 2017
Accepted 21 July 2017
Available online 23 July 2017

Keywords:
Wild raptors
Celioscopy
Morphometry
Histology
Male gonad

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.

© 2017 Elsevier Inc. All rights reserved.

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
very limited and little is known about their sexual maturation, their
gonadal cycle and spermatogenetic activity, both in juveniles and
adult subjects [10]. Usually, at Italian latitude, wild birds are sea-
sonal 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 guar-
ante 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 repre-
sents a period of rest in preparation for the next breeding season
nation, Regression and Resting. During the Regression phase invol-
utive 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 spermatagonia and
few primary spermatocytes [11]. The removing of all degenerating
cells is carried out by macrophages, transiently invading the semi-
iferous 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
rectal and exterior stimuli [11]. In the culmination phase, on the contrary, a male bird is morphologically and functionally ready to undertake reproduction, with sper-
matogenesis 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 charac-
teristics, 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 Univer-
sity 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 en-
dangered 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 Permis) 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
(thr 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 inac-
tive 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 caliper 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 plane 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 con-
ditions 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
دریافت فوری متن کامل مقاله

امکان دانلود نسخه تمام متن مقالات انگلیسی
امکان دانلود نسخه ترجمه شده مقالات
پذیرش سفارش ترجمه تخصصی
امکان جستجو در آرشیو جامعی از صدها موضوع و هزاران مقاله
امکان دانلود رایگان ۲ صفحه اول هر مقاله
امکان پرداخت اینترنتی با کلیه کارت های عضو شتاب
دانلود فوری مقاله پس از پرداخت آنلاین
پشتیبانی کامل خرید با بهره مندی از سیستم هوشمند رهگیری سفارشات