



Investigations on reproductive physiology in the male Eurasian lynx (*Lynx lynx*)

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Abstract

This study characterized (in vivo) morphological and functional parameters of reproductive organs of adult male lynx ($n = 3$) prior to, during, and after the breeding season ($n = 3$). Size and morphology of the reproductive tract were monitored by transcutaneous (testes) and transrectal (accessory sex glands) ultrasonography. Semen was collected by electroejaculation. Ejaculate volume, sperm number, motility, and morphology of spermatozoa as well as testosterone concentrations in blood serum and feces were evaluated. The testes and prostate had seasonal changes in size and echotexture. The mean (\pm S.D.) maximum and minimum testicular volume were $2.8 \pm 0.8 \text{ cm}^3$ and $1.5 \pm 0.3 \text{ cm}^3$, respectively. Fecal testosterone concentrations were highest in February ($1240 \pm 393 \text{ ng/g feces}$), with a second increase in May ($971 \pm 202 \text{ ng/g feces}$), but concentrations were lowest in January ($481 \pm 52.9 \text{ ng/g feces}$). Ejaculate volume, total sperm number and percentage of motile, and intact spermatozoa were maximal in March (the middle of the breeding season). In one of the eight litters, multiple paternity was proven; however, in the remaining seven litters, all 16 cubs were sired by the same male. This particular male had the most developed and active testes and best semen quality, which may be important for sperm competition.

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1. Introduction

The Eurasian lynx (*Lynx lynx*) is distributed from the Northern part of Scandinavia up to the Southern boundary in Turkey and from the European region of the former USSR in the East to the Eastern part of France [1]. However, in the central part of Europe (including Germany), lynx do not exist in stable independent, large populations, in spite of several efforts to re-introduce them into the wild [2]. Knowledge regarding lynx

reproductive biology is limited; most data are derived from carcasses collected from trappers. Data regarding semen quantity and quality in relation to changes in size and activity of the testes and accessory glands contribute to the understanding of male reproductive biology, but so far are not available for lynx. Recent developments in ultrasonography provide the means for non-invasive exploration of the reproductive anatomy and assessment of reproductive health in live male and female lynx [3].

The objective of this study was to characterize the quantity and quality of semen in relation to size and echotexture of the external and internal reproductive organs, as well as testosterone concentrations in serum and feces in live animals, throughout the seasonal cycle.

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These data will be combined with a behavioral and molecular study addressing sexual selection and breeding success.

2. Materials and methods

2.1. Animals

Three adult male lynxes were examined prior to (November), during (March), and after (April and June) the breeding season (between 2002 and 2004). They were housed together with 10 females within six enclosures, each 74 m² and in one large enclosure (10,000 m²) that was fenced and was part of the natural mixed forest providing semi-natural conditions for the lynx. The field research station is situated 50 km northeast of Moscow, surrounded by the natural vegetation of the Russian South Taiga forests. The enclosure design allowed separation of males from females for creation of breeding pairs, providing optimal conditions for behavioral and mating experiments. All animals were well accustomed to humans and apparently did not change their normal behavior during observation from special shelters [4].

For ultrasound examinations of the genital tract and electroejaculation, the animals were immobilized by i.m. administration (via a blow pipe) of 3.5–4.0 mg/kg xylazine hydrochloride (Rompun 10%; Bayer, Leverkusen, Germany) and 3.0–3.5 mg/kg ketamine hydrochloride (Ketamine 10%; Essex, Munich, Germany). After the animals were examined and samples collected, anesthesia was antagonized with 0.2 mg/kg atipamezol hydrochloride (Antisedan; Pfizer, Karlsruhe, Germany).

2.2. Ultrasonography of testes and accessory glands

To ensure acoustic coupling of the ultrasound waves and good image quality, an enema was given and the scrotum was shaved prior to transrectal and transcutaneous ultrasound examination, respectively. Accessory glands (prostate) were visualized by sonography using a transrectal adaptor, as previously described for other medium-sized mammals [3]. The testes and epididymis were imaged by transcutaneous ultrasound, using a hand-held 7.5 MHz curved linear transducer. The dimensions of the testes and prostate were measured ultrasonographically using calipers integrated into the ultrasound machine (EUB 405, Hitachi, Tokyo, Japan). The volume of both organs was calculated according to the volume of a simple rotation ellipsoid, taking into account the spherical and symmetrical shape of these organs.

2.3. Electroejaculation and semen evaluation

Semen collection was performed by electrostimulation under general anesthesia. The method was described previously in roe deer, with slight modifications [5]. Briefly, a rectal probe (2.7 cm in diameter and 8 cm long) with three raised longitudinal electrodes was placed into the rectum above the prostate. Ten electrical stimulations were performed using the Seager model 14 (Dalzell USA Medical Systems, The Plains, VA, USA). The current intensity was increased successively (three stimulations at 20 mA, three stimulations at 50 mA, and the last stimulation at 100 mA). The maximal voltage applied was 5 V. The penis was extracted from the prepuce and massaged gently throughout stimulation. Semen was collected in sterile 2 mL vials (Eppendorf, Hamburg, Germany). Ejaculates were assessed for volume, concentration, motility, and sperm integrity as described before [5].

2.4. Estimation of fecal testosterone by EIA

Feces were collected weekly and monthly during and outside the breeding season, respectively. Testosterone was extracted from the fecal samples [6] and measured by enzyme immunoassay with a double-antibody technique, as described previously [5]. The testosterone concentrations are presented in ng/g feces. Means (\pm S.D.) were calculated for each month. Serial dilutions of a fecal pool gave parallelism to the standard testosterone with no differences in slopes ($P > 0.05$). Inter- and intra-assay coefficients of variation for two biological samples were 12.3% ($n = 11$) and 9.0% ($n = 8$), respectively.

2.5. Mating experiment and paternity analysis

All three males were paired with females ($n = 10$) in a distinct order during the breeding seasons in March 2002 and 2003. During estrus, the female was paired for 4 h with the first male, and on the next day for 4 h with the second one. Mating activities were recorded by permanent observation. “Male–female-combinations” and “order of males” (first or second mating partner) were randomly chosen to test all possible pairing combinations. The design of these mating experiments simulated mating customs of lynx in the wild, where females mate with more than one male during their estrous period and males switch frequently. To determine paternity, DNA-paternity analysis was performed (in 19 cubs from eight litters) by microsatellite analysis, using previously published cat-specific primers [7].

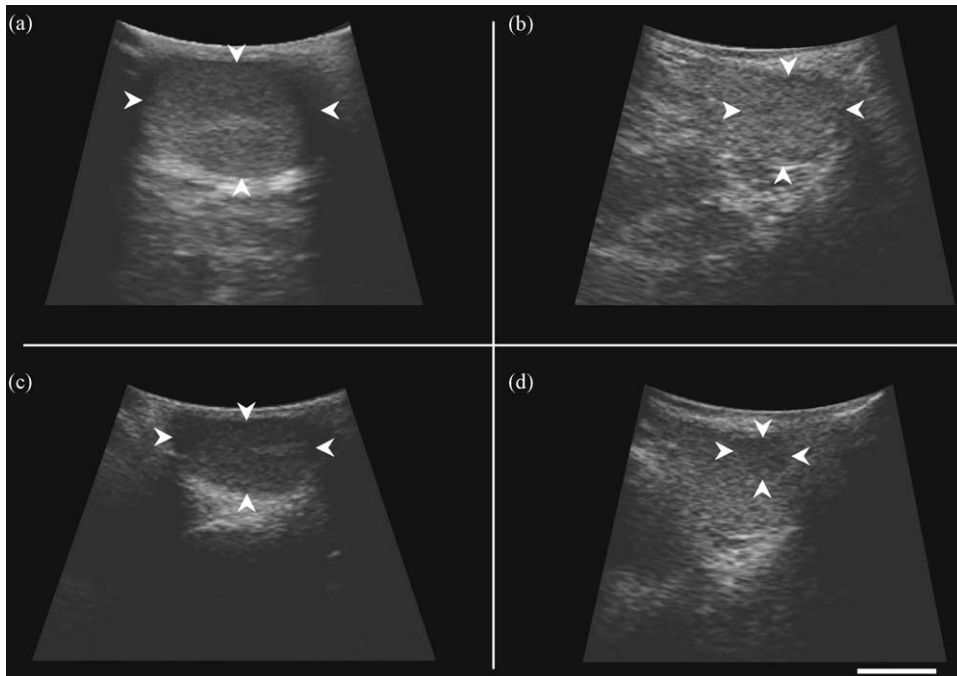


Fig. 1. Sonograms of testis (a and c) and prostate (b and d) of a single male Eurasian lynx during breeding season (March; a and b) and out of breeding season (November; c and d). Note distinct changes in size of the testis and prostate and texture of parenchyma. Arrow heads indicate organ borders. Scale bars represent 1 cm.

3. Results

The ultrasound investigations revealed seasonal changes of the reproductive organs. The testes and prostate had apparent changes in size and texture (Fig. 1); they were most developed in March and June (based on ultrasonography), with a distinct parenchyma and rete testes, whereas in November, they appeared less prominent and not distinguishable. Seasonal changes were most pronounced in testicular size, with a mean maximum and minimum volume of $2.8 \pm 0.8 \text{ cm}^3$ in March and $1.5 \pm 0.3 \text{ cm}^3$ in November, respectively. Fecal testosterone concentrations were highest in February ($1240 \pm 393 \text{ ng/g feces}$), with a second increase in May ($971 \pm 202 \text{ ng/g feces}$), but concentrations were lowest in January ($481 \pm 52.9 \text{ ng/g feces}$; Fig. 2). Ejaculate volume, total sperm number, and percentage of motile and intact spermatozoa were also maximal in March. The ejaculate volumes of all three males were characterized by severe teratozoospermia throughout the year (Table 1).

Mating of females ($n = 10$ (2002) and $n = 8$ (2003)), each with two of the three adult males (in varying combinations and order) resulted in six litters with one to four cubs in both breeding periods. In eight litters with a total of 19 cubs (9 males and 10

females) tested for paternity, there was only one case of multiple paternity (litter sired by “L2”, 2 cubs, and “L3”, 1 cub). However, all 16 cubs in the remaining seven litters were sired from the same male (“L2”) independent from being the first or second mating partner of the respective female. This particular male had the most developed (size) and active (texture) reproductive tract and also the best semen quality (Table 1).

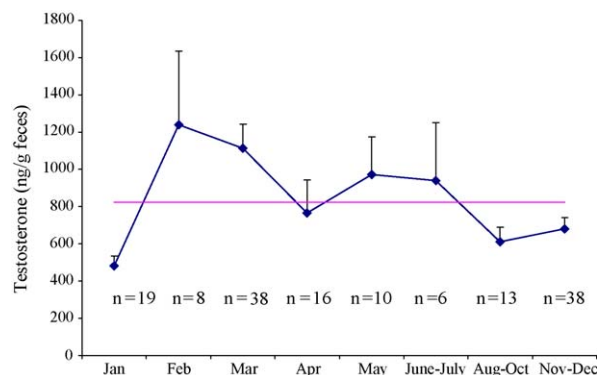


Fig. 2. Testosterone metabolites (mean \pm S.D.) determined in feces of three male Eurasian lynxes over 2 years. Straight line indicates the mean value of all samples ($n = 145$).

Table 1

Mean (\pm S.D.) characteristics of semen collected by electroejaculation from three Eurasian lynxes obtained before (November), during (March) and after breeding season (April and June)

| Animal | Parameter | March | April | June | November |
|--------|------------------------|--------------------|-------------------|-------------------|-------------------|
| L1 | Volume (μ L) | 470 | <10 | <20 | 20 |
| | Density (sperm/mL) | 0.05×10^6 | n.d. | 3.5×10^6 | no |
| | Total number of sperms | 0.2×10^5 | no | 0.7×10^5 | no |
| | Motility (%) | 0 | n.d. | n.d. | n.d. |
| | Intact sperm (%) | 4 | n.d. | 10 | n.d. |
| L2 | Volume (μ L) | 260 | 15 | 30 | 105 |
| | Density (sperm/mL) | 9.5×10^6 | 6.5×10^6 | 110×10^6 | 65×10^6 |
| | Total number of sperms | 25×10^5 | 0.9×10^5 | 33×10^5 | 6.8×10^5 |
| | Motility (%) | 90 | 40 | 60 | 55 |
| | Intact sperm (%) | 29 | 2 | 1 | 13 |
| L3 | Volume (μ L) | 180 | 30 | 10 | 110 |
| | Density (sperm/mL) | 16.6×10^6 | 53×10^6 | 54×10^6 | 10×10^6 |
| | Total number of sperms | 30×10^5 | 16×10^5 | 5.4×10^5 | 11×10^5 |
| | Motility (%) | 90 | 5 | 40 | 5 |
| | Intact sperm (%) | 17.5 | 1.5 | 7.5 | 2 |

n.d.: not determined.

4. Discussion

In contrast to most of the other feline species, the lynx has a relatively short breeding season (March), with parturition in May and June. However, if a female loses the litter for any reason during pregnancy or shortly after parturition, she can come into estrus again in June or July and give birth to a second litter in August or September [8]. This second phase of sexual activity in the female may lead to a corresponding activation of the male in order to ensure reproduction, as demonstrated in the present study by the second increase of testosterone concentration in May and of testicular size in June.

In general, all males investigated ejaculated high proportions of pleiomorphic sperm, which indicated that the lynx is also affected by teratozoospermia, as described for other feline species [9]. Because the animals studied were not inbred and kept under semi-free conditions, we suggest that an incidence of teratozoospermia might be a result of environmental influences (e.g., conditions in captivity and/or poly-male mating system of cats) rather than genetically based. The male “L2” which sired the most offspring had the most developed and activated reproductive tract and also the best semen quality; the latter may be important for sperm competition.

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