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Comparative analysis of intraluteal steroidogenic enzymes emphasises the functionality of fresh and persistent *corpora lutea* during pro—and metoestrus in the *lynx*



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ABSTRACT

European lynx species demonstrate an atypical ovarian cycle compared to other felids. The physiological persistence of corpora lutea (CLs), reflected in constantly elevated progesterone (P4) concentrations in serum, is thought to ensure a seasonal monooestrus. Moreover, the coexistence of CLs from a recent ovulation (freshCLs) and persistent CLs from previous years (perCLs) on the same ovary has been proven. We assume that perCLs in lynxes occur due to fundamentally different mechanisms of luteal regression. Our study presents a detailed analysis of steroidogenic enzymes and steroids in fresh and perCLs obtained from Iberian lynxes during metoestrus, and in perCLs obtained from Eurasian lynxes during procestrus. By quantitative PCR we measured relative mRNA amounts of steroidogenic acute regulatory protein (STAR), cytochrome P450 oxidases (CYPs), hydroxysteroid dehydrogenases (HSDs) and a steroid reductase (SRD). Protein expression in CLs was investigated for CYP11A1, CYP17A1, CYP19A1 and HSD3B. Additionally, the intraluteal and serum steroid content was determined. During metoestrus, mRNA amounts of STAR, CYP11A1, CYP19A1, HSD17B7 and SRD5A1 were significantly higher in perCLs compared to freshCLs. Protein of CYP11A1 was detected independently of the CL age in metoestrus, but expression was less evident in procestrous perCLs. The protein signal of CYP17A1 was strong in freshCLs and perCLs of metoestrus, but weak at procestrus. The presence of CYP19A1 protein was confirmed in each stage of the CL. These findings contribute to the hypothesis that CLs from previous years might support freshly developed CLs for pregnancy maintenance. However, initiation of ovulation might require a functional down-regulation of perCLs prior to breeding. It is noteworthy that the HSD3B1 mRNA amount was significantly elevated in fresh compared to perCLs (metoestrus). Accordingly, HSD3B protein was substantially present in freshCLs, whereas signals were literally absent in all perCLs. Elevated expression of HSD3B coincided with high intraluteal oestrogen concentrations in freshCLs; however, the enzyme pattern was less concordant with intraluteal P4 and androgen concentrations. Serum P4 concentrations of Iberian lynxes were constant between procestrus and prolonged dioestrus. Moreover, constantly high serum oestrogen concentrations were measured during pro-, met- and prolonged dioestrus. The physiology of exceptionally high serum oestrogen concentrations outside the breeding season of lynxes merits further investigation. In conclusion our study supports the concept that the unique reproductive strategy of lynxes is directly linked to sustained intraluteal steroid biogenesis in persistent CLs. © 2015 Elsevier Ltd. All rights reserved.

Abbreviations: CL, corpus luteum; CYP, cytochrome P450 oxidase; EL, Eurasian lynx; freshCL, freshly formed CL; HSD, hydroxysteroid dehydrogenase; IL, Iberian lynx; P4, progesterone; perCL, persistent CL; SRD, steroid reductase; STAR, steroidogenic acute regulatory protein.

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1. Introduction

The lvnx genus consists of four species of short-tailed cats (family *Felidae*) found in the forests of Europe, Asia and North America. The Eurasian lynx (Lynx lynx Linnaeus, 1758), the Canada lynx (L. canadensis Kerr, 1792) and the bobcat (L. rufus Schreber, 1777) are considered as *least concern* by the International Union for Conservation of Nature and Natural Resources (IUCN), whereas the Iberian lvnx (L. pardinus Temminck, 1827) is declared as critically endangered [1]. Therefore, various attempts to protect this species from extinction have been initiated. An integrated species conservation plan links the in situ (Iberian Lynx Conservation Breeding Programme, ILCBP; Portugal, Spain) with the ex situ (Lince Andalucía–Population recovery of Iberian Lynx in Andalusia, EU LIFE Project) conservation efforts. The main goals of the ILCBP are to maintain a genetically well-managed captive population, supporting wild populations by lynx re-introduction [2-4]. Furthermore, the ILCBP provides the basis for research on reproductive physiology and associated technologies [5].

Captive population management of *Iberian* and *Eurasian lynxes* is already supported by the tools of pregnancy diagnosis and parturition prediction [6,7]. Moreover, non-invasive monitoring of hormone metabolites [8,9], as well as studies on steroid metabolism [10,11], have documented endocrine profiles of reproduction in female *lynxes*. As a central reproductive peculiarity, Iberian and *Eurasian lynxes* exhibit a non cat-like ovarian cycle, as shown by ultrasonographical and endocrinological analyses [12]. The Canada, the Iberian and the *Eurasian lynx* all reveal a strict seasonal monooestrous cycle [2,8,13,14] in contrast to the polyoestrous reproductive patterns of most other felids [15] including the bobcat [16,17] and the domestic cat (*Felis silvestris forma catus* Linnaeus, 1758; [18]).

As there exist no confounding species differences regarding reproduction, except for a moderate shift in the beginning of the mating season (Jan–Apr for *Eurasian lynx* [14], Jan–Feb for *Iberian lynx* [2]), the *Eurasian lynx* is commonly used as a model species to gain knowledge of the reproductive physiology of the critically endangered *Iberian lynx* [10,12,19]. Recently, *intra-vitam* longitudinal ultrasound studies of *Eurasian lynx* ovaries, supported by information on serum steroid concentrations, revealed the physiological persistence of *corpora lutea* (CLs) [19]. These

persistent CLs are preserved structurally for at least two subsequent years and are assumed to be responsible for the almost constantly elevated progesterone (P4) concentrations in serum throughout the year, thus preventing ovulation and, presumably, ensuring the monooestrous cycle [12,19]. The observation that central and northern European populations of both captive and free-ranging Eurasian lynxes all show the same reproductive strategy suggests that there is no plasticity regarding this phenomenon [19]. The only difference was found concerning the beginning of the breeding seasons (oestrus occurred at the end of February until mid-March for captive lynxes and at the end of March for free-ranging lynxes), which is most likely latitude, and therefore delayed photoperiod, dependant [19]. Since luteal activity is observed outside the breeding season, in contrast to the typical anoestrus period of other felids, this activity was referred to as prolonged dioestrus (for a detailed description of the reproductive stages of the *Eurasian lynx* cycle see [19]). Histological characterisation and determination of intraluteal steroid content in Eurasian lynx further established the coexistence of freshly formed CLs of a recent ovulation (freshCLs) and persistent CLs of previous years (perCLs) on the same ovary [20-22]. Despite annual luteal activity, female lynx can regularly enter oestrus, and gestation is terminated by parturition, which is supposedly ensured by temporary functional regression of CLs prior to the onset of oestrus or near term [19].

In most mammals, CLs functionally and structurally regress at the end of the luteal phase in a process called luteolysis [23]. In pregnant and non-pregnant domestic cats, *e.g.* the structural transition from CL formation to the *corpus albicans* [24,25] correlates with decreasing plasma P4 concentrations [26–28]. Since this is reflected by an ongoing loss of intraluteal steroidogenic capacity [29,30], intraluteal steroids are potential factors for assessing luteal function in felids.

Regulation of *de novo* steroid biogenesis requires mobilisation and transfer of cholesterol by the steroidogenic acute regulatory protein (STAR) [31]. Basic pathways of conventional and alternate steroid biogenesis and steroid metabolism comprise conversion of cholesterol to pregnenolone, metabolism of pregnenolone into various intermediates and active steroids, and metabolism of steroid precursors and steroids [32] (Fig. 1). Most steroidogenic enzymes belong to cytochrome P450 oxidases, such as the

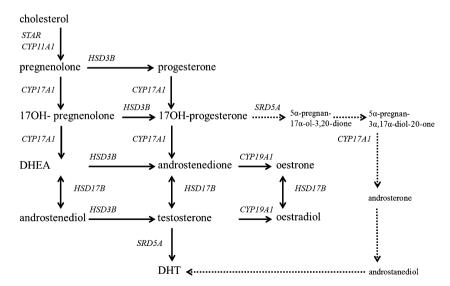


Fig. 1. Conventional (—) and alternate (--) pathways of steroid biogenesis. STAR (steroidogenic acute regulatory protein), CYP11A1 (cholesterol side-chain-cleavage enzyme), HSD3B (3 β -hydroxysteroid dehydrogenase/ $\Delta^5 \rightarrow \Delta^4$ isomerase), CYP17A1 (steroid 17- α -monooxygenase), CYP19A1 (P450 aromatase), HSD17B (17 β -hydroxysteroid dehydrogenase), SRD5A (steroid 5- α -reductase), DHEA (dehydroepiandrosterone), DHT (dihydrotestosterone). Reproduced with permission from J Steroid Biochem Mol Biol, 2014. 144(Part B): p. 373–81 [29].

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