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Progesterone, estrogen, and androgen receptors in the *corpus luteum* of the domestic cat, Iberian lynx (*Lynx pardinus*) and Eurasian lynx (*Lynx lynx*)

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ABSTRACT

In contrast to the species studied, the *corpus luteum* (CL) of Iberian and Eurasian lynx physiologically persists in the ovary for more than 2 years and continues to secrete progesterone. Such persistent CL (perCL) transition into the next cycle and are present in the ovary together with the freshly formed CL (frCL) of a new ovulation. To date, the mechanisms supporting such CL persistence are not known. We analyzed the potential receptivity of feline CL to sex steroids through mRNA measurements of progesterone receptor (PGR), progesterone receptor membrane components (PGRMC) 1 and 2, estrogen receptor (ESR) 1 and ESR2, G protein–coupled estrogen receptor 1 (GPER1), and androgen receptor (AR). All receptors were present in domestic cat CL during pregnancy and the nonpregnant luteal phase, in frCL and perCL of post-mating Iberian lynx and in perCL of pre-mating Eurasian lynx. Mass spectrometry detected the presence of PGRMC1 protein in frCL and perCL of the Iberian lynx. In both domestic cat and lynx CL, PGR, PGRMC1, and ESR1 proteins were localized in luteal cells by immunohistochemistry. The mRNA levels of *PGR*, *PGRMC1*, *PGRMC2*, *ESR1,* and *AR* changed significantly throughout the domestic cat luteal phase. This may indicate involvement of these receptors in the processes of formation, maintenance, and regression of feline CL. In Iberian lynx, expression of *PGRMC1*, *PGRCM2*, *ESR1*, *GPER1,* and *AR* was significantly higher in perCL compared with frCL, whereas *ESR2* was reversed. High mRNA amounts of these receptors in perCL suggest that physiological persistence of lynx CL may be partly regulated by actions of sex steroids through their nuclear and/or membrane receptors.

1. Introduction

Reproductive patterns vary widely among species and are heavily dependent on the function of the *corpus luteum* (CL), a transient gland that forms in the ovary after ovulation and maintains pregnancy in many species through its production of progesterone [1]. In all mammals studied so far, including the domestic cat (*Felis catus*), CL regress from ovarian tissue at the end of pregnancy or in the nonpregnant luteal phase, allowing the initiation of a new cycle. The situation, however, is markedly different for another member of the *Felidae* family, the lynx. Studies on the *Lynx* genus have revealed that Iberian and Eurasian lynx

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(*Lynx pardinus* and *Lynx lynx*, respectively) exhibit a non–cat-like ovarian cycle, in which CL physiologically persist in the ovary for more than 2 years and remain functionally active in their production of steroids [2–4]. The mechanisms underlying such physiological persistence of CL are not clear, and studies to unravel this reproductive peculiarity are only just beginning.

The domestic cat has been considered as seasonally polyestrous [5]. After induced or spontaneous ovulation, queens can either enter a period of pregnancy (~65 days [6]) or a nonpregnant luteal phase (~40 days [7]). In both scenarios, elevated serum progesterone decreases toward the end of the luteal phase, allowing initiation of a subsequent ovarian cycle [8]. The intraluteal concentration of progesterone and the capacity of CL to produce it also decline by the end of pregnancy and the nonpregnant luteal phase [9,10]. In the domestic cat, CL are the main source of progesterone, and the placenta is a supplemental site of synthesis [11,12].

The *Lynx* genus includes four species: the Eurasian lynx, the Iberian lynx, the Canada lynx (*Lynx canadensis*), and the bobcat (*Lynx rufus*). In contrast to the domestic cat, all lynx species, excluding the bobcat, are identified as monoestrous [13]. In the three monoestrous species, functional activity of persistent CL (perCL) was confirmed outside pregnancy and weaning [2,4,14]. In bobcats, CL persist morphologically, yet their functional activity has not been investigated [15,16]. We hypothesize that one of the roles of perCL in lynx is to secure a monoestrous cycle through suppression of ovarian activity, which would ensure the birth and weaning of cubs during the most favorable time of the year [3].

The steroid hormones progesterone, estrogens, and androgens play an essential role in female reproduction, including formation, maintenance, and regression of the CL, and CL themselves have the capacity to express steroid receptors [17–19]. Progesterone actions can be transduced in the CL through its nuclear receptor progesterone receptor (PGR), membrane progestin receptors, and progesterone receptor membrane components (PGRMC) 1 and 2 [20]. Estrogen has at least two nuclear receptors, estrogen receptors (ESRs) 1 and 2 (also known as ESR alpha and beta, respectively), the yet to be determined membrane receptors and the recently discovered G protein–coupled estrogen receptor 1 (GPER1) [17,21,22]. Finally, the androgen receptor (AR) has been identified in CL tissue of pigs and rodents [19,23,24].

The PGR, ESR1, ESR2, and AR belong to the nuclear receptor superfamily and act as hormone-dependent transcriptional factors. After binding to the ligand and subsequent release from heat shock proteins, receptors undergo translocation to the nucleus and dimerization, and with the recruitment of co-factors, they regulate gene transcription on specific sequences of DNA [25]. Such signal transduction through nuclear receptors is called the classical genomic pathway, and the resultant effect can be delayed for hours. Additionally, a nongenomic pathway of rapid signaling exists through membrane receptors.

To our best knowledge, there is yet no information on the receptivity to steroid hormones of the feline CL, including the model felid, the domestic cat. To fill this gap, we focused on the detection of mRNA for nuclear and membrane receptors of progesterone (*PGR*, *PGRMC1*, and *PGRMC2*), estrogen (*ESR1*, *ESR2*, and *GPER1*), and androgen (*AR*) in the CL tissue of the domestic cat and on the changes in its levels throughout pregnancy and the nonpregnant luteal phase. Moreover, we analyzed the relative mRNA amounts of these receptors in perCL of Iberian and Eurasian lynx and in freshly formed CL (frCL) of the Iberian lynx to learn more about the uncommon features of lynx reproduction. The knowledge obtained here could contribute to future studies on feline reproduction, assist in the development of reproductive techniques for the Iberian lynx (endangered species [26]), and advance our understanding of the physiology of CL persistence in general.

2. Materials and methods

All chemicals in the study were purchased from Sigma–Aldrich (Taufkirchen, Germany), unless stated otherwise and were of the highest purity available. The methods applied, and the study design, were approved by the Internal Committee for Ethics and Animal Welfare of the Leibniz Institute for Zoo and Wildlife Research in Berlin, Germany (Permit numbers: 2010–10–01 and 2011–01–01).

2.1. Animals and tissue collection

Ovaries of domestic cats were obtained from local animal shelters and clinics after ovariohysterectomy for the purpose of permanent contraception. Samples were transported in the Minimum Essential Medium Eagle HEPES, supplemented with 3 mg/mL BSA (Merck Millipore, Darmstadt, Germany) and 1× antibiotic antimycotic solution. Transportation was at 4 °C, and ovaries were processed immediately after arrival at the laboratory (2–4 hours after surgery). The isolation and consequent staging of CL are described in Amelkina et al. [10]. In brief, CL from each cat were either fixed in Bouin's solution for histologic analysis or plunged into liquid nitrogen for RNA isolation. In the case of pregnancy, the day was assessed by the diameter of the gestation chamber [27], the crown-rump length of a fetus [28], or by the stage of preimplantation embryos [29]. The preimplantation period (n = 6) included samples from Days 2 to 6 and 10 *post-coitum* (implantation in the domestic cat starts between Days 13 and 15 *post-coitum* [29]); the postimplantation period (n = 11) included samples from Days 14 to 36; finally, the CL regression stage (n = 5) was represented by samples from Days 38, 39, and 48 and Week 9. The absence of embryos in the oviducts or uteri indicated a nonpregnant luteal phase. In such cases, based on their histologic appearance, each CL was classified as the stage of formation (n = 9), development/maintenance (n = 13), early regression (n = 14), late regression (10), or *corpus albicans* (n = 4). The histologic classification is described in detail in Amelkina et al. [10] and includes parameters of cell shape, type and degree of vacuolation, nucleus condition, and the ratio of nonsteroidogenic to luteal cells. Listed n values represent the number of animals per analyzed stage; each animal is represented by one CL.

Ovaries of Iberian lynx were collected within the scope of the Iberian lynx conservation breeding program at the Centro de Cría de Lince Ibérico El Acebuche, Parque Nacional