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Corpora lutea of pregnant and pseudopregnant domestic cats reveal similar steroidogenic capacities during the luteal life span

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ABSTRACT

In domestic cats, luteal phases of pregnancy and pseudopregnancy (non-pregnant luteal phase) differ in the course and level of plasma progesterone (P4). Therefore, we assumed differences in luteal steroidogenic capacities. Here we present a comprehensive analysis of intraluteal steroid biogenesis in the domestic cat. We quantitatively measured relative mRNA levels of steroidogenic acute regulatory protein (STAR), cytochrome P450 oxidases (CYP), hydroxysteroid dehydrogenases (HSD), steroid reductase (SRD) and enzymes involved in sulfoconjugation of steroids, i.e. sulfotransferase (SULT) and sulfatase (STS). Protein expression was analysed by Western Blot for HSD3B. Additionally, intraluteal steroid contents were determined. During the pseudopregnant luteal phase, expression of STAR ($p = 0.005$), HSD3B1 ($p < 0.0001$), CYP19A1 ($p < 0.0001$) and HSD17B7 ($p = 0.008$) decreased from formation of the *corpus luteum* (CL) onwards. HSD3B protein expression was highest in the development/maintenance stage of CL and declined during the subsequent luteal phase of pregnancy and pseudopregnancy. This was in accordance with decreasing intraluteal levels of P4, oestrogens and androgens. In contrast, expression of SRD5A1 ($p < 0.001$) increased with progression through stages of the pseudopregnant CL, being indicative of P4 metabolism via an alternate pathway to dihydrotestosterone (DHT). Compared to the formation stage, expression of SULT1E1 was higher in all other luteal stages of pseudopregnancy ($p = 0.004$), implying a potential sulfoconjugation of oestrogens. Expression of CYP11A1 and CYP17A1 was unaffected by the luteal stage ($p > 0.05$), suggesting a permanent capacity of cat CL to convert progestogens via androgen and oestrogen pathways. In general, mRNA expression profiles of steroidogenic enzymes during the pregnant luteal phase reflected the pseudopregnancy profiles. Intraluteal oestrogen ($p < 0.0001$) and androgen ($p = 0.008$) levels were higher in the formation stage compared to the following luteal stages of pseudopregnancy. Concentrations of P4 were higher in the development/maintenance compared to the regression stages ($p = 0.01$). We conclude that cat CL of the same histomorphological stage are characterised by identical steroidogenic capacities independently of an on-going pregnancy.

1. Introduction

Domestic cats (*Felis silvestris* forma *catus* Linnaeus, 1758) exhibit a coitus-induced luteal phase [1,2], although spontaneous ovulation without cervical or vaginal stimulation was confirmed as well [3,4]. The luteal phase in domestic cats is characterised by plasma progesterone (P4) elevations, reflecting the formation of functional *corpora lutea* (CL) on the ovary. Depending on the mating success, two types of luteal phases exist: one associated with pregnancy and a sterile luteal phase, often referred to as pseudopregnancy. The plasma P4 and oestradiol (E2) profiles during pregnancy and the pseudopregnant luteal phase have been

Abbreviations: CA, *corpus albicans*; CL, *corpus luteum*; CYP, cytochrome P450 oxidase; d/m, development/maintenance; DHT, dihydrotestosterone; E2, oestradiol; er, early regression; f, formation; HSD, hydroxysteroid dehydrogenase; lr, late regression; P4, progesterone; PP, pseudopregnancy; PR, pregnancy; SRD, steroid reductase; STAR, steroidogenic acute regulatory protein; STS, sulfatase; SULT, sulfotransferase.

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extensively investigated by blood sampling [1,5–9]. Within the first five days after mating, plasma E2 abruptly declines from preovulatory peak values to baseline and only slightly rises prior to parturition [5,6]. The levels of plasma E2 during pseudopregnancy are in a similar range compared to pregnancy [5]. The origin of preimplantative and prepartal E2 elevations remained unexplained, but follicular and luteal cells have been discussed as possible sources [1]. Plasma P4 profiles of pregnant and pseudopregnant cats proceed similarly until day 10–12 *post coitum* (*p.c.*) coinciding with time point of implantation [10]. Thereafter, plasma P4 levels are considerably higher in pregnant females, reaching peak values at day 21, followed by a constant decrease until parturition at day 65 ± 4 [5]. In the pseudopregnant luteal phase, plasma P4 already becomes basal after 40–45 days [7]. Assuming that plasma P4 levels reflect functional luteal phases in cats, the pseudopregnant phase lasts about two thirds of the respective gestational period. Shortened luteal phases have already been documented in pseudopregnant rabbits [11] and rats [12]. Other carnivores, like mink [13] and ferret [14], reveal luteal phases of pregnant and pseudopregnant females with similar lengths, whereas in the dog, CL can retain their function beyond normal time of parturition (80 versus 65 days) [15].

It has long been assumed that placental P4 contributes to the variation in plasma P4 levels in pregnant versus pseudopregnant cats. The placental capacity to convert pregnenolone to P4 near term [16], as well as intraplacental P4 content and expression of steroidogenic enzymes, have been subsequently proven [17,18]. However, differences between plasma P4 profiles in pregnant and pseudopregnant cats remained unexplained, as placental steroid profiles do not reflect plasma levels of P4 and E2 [17]. Furthermore, ovariectomies lead to a rapid decrease of plasma P4 and in some cases to abortion [8,9,19], providing evidence for luteal origin of plasma P4. Mechanisms for pregnancy maintenance after ovariectomy are still unknown, but might be supported by local P4 production by the feline placenta.

According to histomorphology and steroid hormone levels, the luteal tissue of cats undergoes severe transformations with progression through the stages of formation, development/maintenance, regression and the *corpus albicans* [20]. Little is known, however, about the underlying mechanisms of steroidogenic transformations during the luteal life span in cats. Generally, the conversion of cholesterol to active steroids within steroidogenic cells is catalysed by steroidogenic enzymes. Steroidogenic enzymes belong mainly to cytochrome P450 oxidases (CYP) and hydroxysteroid dehydrogenases (HSD); the steroid reductases (SRD) are unrelated to these families (Fig. 1; for review see Miller [21] and Ghayee and Auchus [22]).

The *de novo* steroid biogenesis requires transport of cholesterol through the mitochondrial membrane by the steroidogenic acute regulatory protein (STAR) [23]. Cholesterol is then converted to pregnenolone by the cholesterol side-chain-cleavage enzyme (CYP11A1). The oxidation of Δ^5-3β-hydroxysteroids to Δ^4-3-ketosteroids (e.g. pregnenolone to P4, androstenediol to testosterone) is catalysed by 3β-hydroxysteroid dehydrogenase/$\Delta^5 \rightarrow \Delta^4$ isomerases (HSD3B). A steroid 17-alpha-monooxygenase (CYP17A1) converts pregnenolone and P4 to intermediates for testosterone and oestrogen synthesis. C19-androgens are aromatised to C18-oestrogens by P450 aromatase (CYP19A1). Different 17β-hydroxysteroid dehydrogenases (HSD17B) convert androstenedione to testosterone, as well as oestrone to E2, and vice versa [24]. Steroid-5-alpha-reductase isozymes (SRD5A) are involved in steroid metabolism by converting 3-oxo (3-keto), $\Delta^{4,5}$ C19/C21 steroids to 5α-stereoisomers, e.g. testosterone to dihydrotestosterone (DHT) [25] and progesterone to 5α-reduced progestogens [26]. Steroid sulphates are derived from sulfonation of steroids by sulfotransferase enzymes, whereby oestrogens are sulfonated by the cytosolic SULT1E1 [27]. The conformational changes of oestrogens by sulfoconjugation can be reversed by hydrolysis of steroid sulphates to native steroids by a sulfatase (STS) [21].

The aim of our study was to provide the first comprehensive analysis of intraluteal steroid biogenesis pathways in the domestic cat. Based on different plasma P4 profiles during pregnancy and pseudopregnancy [5], we assumed differences in luteal steroidogenic capacities. We quantitatively measured relative mRNA levels of steroidogenic acute regulatory protein, cytochrome P450 oxidases, hydroxysteroid dehydrogenases, steroid reductase and enzymes involved in sulfoconjugation of steroids. These data were supplemented by investigating protein expression of HSD3B. The steroid content in different luteal stages was determined as well. This study was conducted to contribute to fundamental knowledge on luteal function and to provide insight into the mechanisms regulating luteolysis in felids.

2. Materials and methods

2.1. Tissue collection

Ovaries and uteri of domestic cats were collected from local animal shelters and clinics after ovariohysterectomy/ovariectomy, whereby time point of ovulation was unknown for each animal. Ovaries were dissected for CL isolation. Day of pregnancy was determined according to (i) the stage of embryos flushed from the oviduct (<day 5 *p.c.*; [28]), (ii) the diameter of the gestation chamber (<day 20 *p.c.*; [29]) or (iii) to foetal crown-rump length (>day 20 *p.c.*; [30]). In absence of embryos or foetuses, a pseudopregnancy was assumed. Stages of pseudopregnancy were established based on the histomorphology of CL (routine haematoxylin/eosin staining) and by comparison to existing literature, as well as to the observed course of pregnancy [20]. Accordingly, CL of pregnant and pseudopregnant cats were allocated to five sequential luteal stages: formation (f), development/maintenance (d/m), early regression (er) and late regression (lr). For pregnancy this included day 2–5 (f), day 14–36 (d/m), day 38–39 (er) and day 48–63 (lr), respectively PP1 (f), PP2 (d/m), PP3 (er) and PP4 (lr) for pseudopregnancy. *Corpora albicantia* (CA) were not assigned to either pregnant or pseudopregnant luteal phases, since they could have been remnants of functional CL after parturition and weaning [31] or of a pseudopregnancy. Due to limitation of luteal tissue, histological, hormonal and molecular analyses were not performed on the same CL. However, comparability was ensured by random selection of CL with same appearance from one ovary for either fixation in Bouin's solution (histological analysis) or in liquid nitrogen (hormone, mRNA and protein studies).

2.2. Sequence analysis

Total RNA was isolated from feline reproductive tissues according to the innuSPEED Tissue RNA/innuPREP DNase I Digest Kit (*HSD17B7*; Analytik Jena AG, Jena, Germany) or the Precellys Tissue RNA/peqGOLD DNase I Digest Kit (*STAR*, *HSD17B2*, *SRD5A1*, *SULT1E1*, *STS*; PEQLAB Biotechnologie GmbH, Erlangen, Germany). Reverse transcription of total RNA into single-stranded cDNA (ss cDNA) was performed with the RevertAid First Strand cDNA Synthesis Kit (Thermo Scientific, Schwerte, Germany). For the polymerase chain reaction (PCR) primers were purchased from BioTeZ Berlin Buch GmbH (Berlin, Germany). Primers were based on predicted (*HSD17B7*) or published (*STAR*) *Felis catus* gene sequences. Sequences of *HSD17B2*, *SRD5A1*, *SULT1E1* and *STS* were not annotated in GenBank at the beginning of the study. Therefore, primers for their amplification were derived from consensus sequences after a multiple species sequence alignment (CLC