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Fecal estradiol-17β and testosterone in prepubertal domestic cats

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ABSTRACT

The aim of this article was to describe the time course of prepubertal sexual steroids in domestic cats. Fourteen newborn kittens were followed up until puberty (physical, behavioral, and hormonal changes). Fecal testosterone [T; males] and E estradiol 17-β [E2; females] concentrations were analyzed by repeated measures ANOVA and two consecutive time windows (TWs) were used to compare changes in both male (postnatal weeks 1–4 vs. 5–14) and females (postnatal weeks 1–5 vs. 6–13). Puberty was achieved 14.3 ± 0.3 and 13.3 ± 0.4 weeks after birth in male and female cats, respectively. In both genders, during TW-1 fecal steroids concentrations were similar (males) or even higher (females) to that previously described for mature cats. Fecal T ($P < 0.01$) and E2 ($P < 0.01$) varied throughout the weeks. Differences were found when hormonal concentrations of TW-1 were compared with those of TW-2 both for male (61.4 ± 7.9 vs. 16.9 ± 2.2 ng/g; $P < 0.01$) and female (78.2 ± 12.5 vs. 11.2 ± 4.0 ng/g; $P < 0.01$) cats. It is concluded that in domestic cats there is a sexual steroid surge during the first 4 and 5 postnatal weeks in male and female animals, respectively.

1. Introduction

At delivery, newborn mammals are subjected to an abrupt withdrawal of maternal hormones and a release of the hypothalamic-pituitary-gonadal axis that is characterized by a significant increase of gonadotropins after birth [1–4]. Then, the neonate gonads are stimulated and respond with elevated sexual hormone production (testosterone (T) and estradiol 17-β (E2) [5]), which will subsequently decline to slowly achieve typical prepubertal values until sexual maturity [1,4,6].

In rats, this active neonatal hormone (i.e., gonadal steroids) environment is crucial for the organization of reproductive function and later sexual maturation [1]. There is, therefore, a critical period of vulnerability in the neonatal stage [7]. Interference with normal pituitary-gonadal function during this time window (TW) impacts adversely on genital tract development and subsequent adult reproductive performance [6]. The precise period of developmental vulnerability is species-specific and it apparently depends on the state of maturity of the animal at the time of birth [8]. In this aspect, domestic carnivores are born in a less mature state than the other domestic species; this event might make them particularly labile during the postnatal period.

Present knowledge about the prepubertal sexual steroids and the critical TW of vulnerability, in which exposure to foreign substances should be avoided, is null in domestic cats. The paucity of feline data may be due to ethical, methodological, and practical difficulties inherent in serial serum sampling in postnatal kittens. In this study, noninvasive fecal steroid assays were used to monitor the development and activation of the gonadal axis in the experimental animals. Thus, the aim of this article was to describe the time course of sexual steroids (T and E2 for males and females, respectively) from birth to puberty in domestic cats (*Felis catus domesticus*).

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2. Materials and methods

2.1. Animals

Fourteen half or full siblings, mixed-bred kittens (seven males and seven females), which were born (mean body weight 110 ± 10 g) in our Institutional cat colony after normal gestation (mean length 65 ± 1 days), were included in this study. The animals were reared free in indoor catteries (2 rooms 4 × 3 m, with 14 hours of light and 10 hours of dark per day, average room temperature 22 °C, average humidity 65%, and appropriate enrichment), weaned at the age of 30 days and fed with dry commercial premium kitten food and water *ad libitum*. The kittens were socialized by a group of trained students. This study was approved by the Faculty Institutional Care and Animal Use Committee (IACUC).

2.2. Experimental design

The kittens were followed up until immediately before puberty when they were gonadectomized and adopted by families. Follow-up included sexual behavior observation (≥1.5 hours twice daily) and fecal samples collection. From the third month after birth, genital examination in males (balano-preputial separation, eruption of penile spines weekly) and vaginal cytology in females (three times a week; [9]) were also carried out.

One hundred and ninety-six fecal samples were weekly collected and frozen for T and E2 determinations in the male and female cats, respectively. For these purpose, individual cats were caged one night a week throughout the study period. Fecal steroids were processed on the basis of the general methods described by Brown et al. [10]. All fecal data were expressed on a wet-weight basis.

In males, puberty was defined as complete balano-preputial separation and the appearance of penile spines, whereas in females by the findings of >80% superficial keratinized cells and a clean background in vaginal smears. In both genders, the expression of the corresponding typical sexual behavior (i.e., rubbing on objects, rolling, lordosis, tail lateralization, and vocalizing in females and mounting in males) was also included as a requirement [9].

2.3. Statistical analysis

Fecal T and E2 concentrations (mean ± SEM) in males and females, respectively, were analyzed by repeated measures ANOVA. The level of significance was set at $P < 0.05$ (SPSS 17.0, SPSS, Chicago, IL, USA).

3. Results

Puberty was achieved 14.3 ± 0.3 and 13.3 ± 0.4 weeks after birth in male and female cats, respectively. Fecal T ($P < 0.01$; Fig. 1) and E2 ($P < 0.01$; Fig. 2) concentrations varied throughout the weeks of the study. When two consecutive TWs were defined and compared for male (postnatal weeks 1–4 vs. 5–14) and females (postnatal weeks 1–5 vs. 6–13), differences were found between hormonal concentrations of TW-1 versus TW-2 both for male (61.4 ± 7.9 vs. 16.9 ± 2.2 ng/g; $P < 0.01$) and female (78.2 ± 12.5 vs. 11.2 ± 4.0 ng/g; $P < 0.01$) animals.

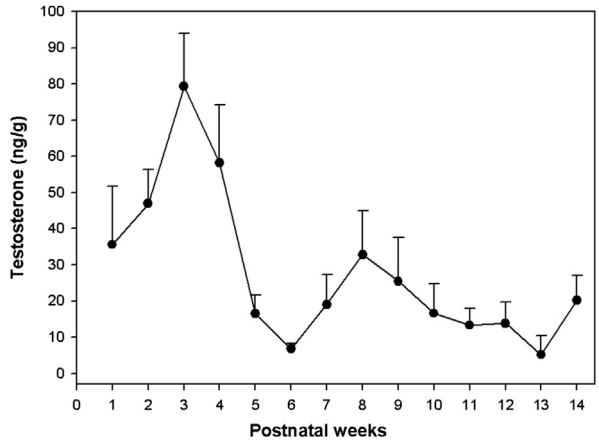

Fig. 1. Fecal testosterone (mean ± SEM) of seven postnatal (0 represents birth) male kittens followed up until before puberty.

4. Discussion

This study is the first documentation of the chronology of postnatal sexual steroids surge in domestic felids. Fecal steroid measurement has been extensively employed in endocrinology of wild and domestic felids [10] being well-suited for use in longitudinal protocols. Furthermore, fecal steroids provide a noninvasive, time-integrated measure over several days [10].

Each gender was studied separately as males and females are known to undergo development at different rates, both *in utero* and postnatally up until the post-pubertal [11]. As expected, in these animals, sexual maturity was reached earlier in females than in males, a situation that is preserved across a range of species from mice to humans [11]. Furthermore, the age of puberty in these animals was consistent with what has been previously reported for the species [9] and also for our colony.

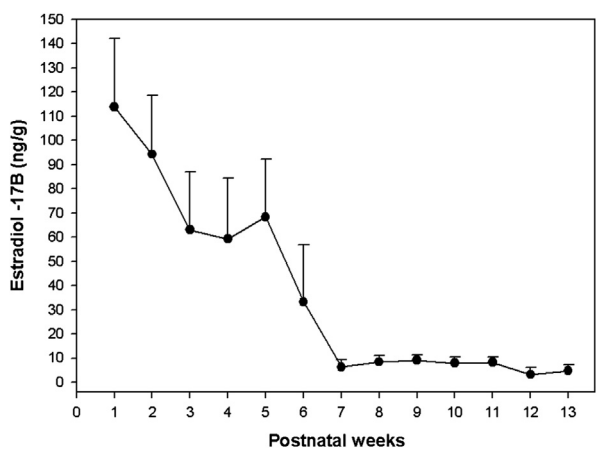

Fig. 2. Fecal estradiol 17-β (mean ± SEM) of seven postnatal (0 represents birth) female kittens followed up until before puberty.

The present results seem to show that domestic cats are not an exception among mammals [3,4], and birth is followed by an increase in sexual hormones. It is noteworthy the high sexual steroid concentrations achieved during the first postnatal weeks in both genders. Either in males and female cats, these concentrations were significantly higher than those of remaining weeks up to puberty. In female kittens, E2 seemed to gradually decrease up to middle of the follow-up period. Furthermore, during the high period, E_2 fecal concentrations were higher than those that we have for estrous queens and T was similar to that of mature intact toms of our cat colony. A similar biphasic (i.e., adult-like followed by low levels) pattern of prepubertal hormone secretion has been documented in rats, monkeys, and humans [1,4,6].

An additional necessary step toward the accurate description of the postnatal hormone surge in cats was to determine when this TW begins and when it is completed in each gender. Postnatal surge seemed to be longer in female than in male cats, that is, 5 versus 4 weeks; this difference in timing of the postnatal surge between genders might show particular roles of this process in each sex. Circulating levels of gonadotropins differ markedly in female and male rats from birth to puberty, and this has been related to different neural components conditioned by early sexual differentiation of the hypothalamus [12]. In humans, gonadotropin surge also differs in character between male and females. In coincidence with the present results, in female infants, the dynamics of the postnatal activation of the gonadal axis is more complex and hormonal levels are more sustained and heterogeneous [4,13].

The timing of the critical period deserves special mention. In the rat, this has been determined to occur during the first few days before and after birth [14]. In primates, plasma T concentrations are elevated for some 3 months from birth [15]. In this aspect, it should be bear in mind that comparisons are difficult as birth occurs somewhat arbitrarily during mammalian development, and species are born in differing stages of somatic and neurobiological maturation [14].

In rodents and primates, the neonatal hormone environment profoundly affects the ultimate sexually differentiated pattern of central nervous system, reproductive physiology, and behavior, as well as immune system development and maturation [1,6,7]. In felids, the physiological significance of postnatal hormonal surge remains to be studied as the simple extrapolation from other species would be speculative. Data presented here will provide critical information needed for future investigations designed to unveil the role of this neonatal “mini-puberty” in the development of sex- biology and behavior. Immediate awareness of the potential consequences of postnatal exposures during the identified TWs of vulnerability should be considered by both clinicians and researchers working with this species. Finally, it is concluded that in domestic cats there is a sexual steroid surge during the first 4 and 5 postnatal weeks in male and female animals, respectively.

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Competing interests

MF and AC are research fellows and CG is a career scientist of CONICET, Argentina. None of the authors of this article has a financial or personal relationship with other people or organizations that could inappropriately influence or bias the content of the article.

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