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Theriogenology 78 (2012) 1224–1234

Theriogenology

www.theriojournal.com

Testicular testosterone: Estradiol ratio in domestic cats and its relationship to spermatogenesis and epididymal sperm morphology

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Received 11 November 2011; received in revised form 13 April 2012; accepted 21 May 2012

Abstract

The phenomenon of teratozoospermia in felids is not fully understood. In this study, we investigated the testicular androgen:estrogen balance in domestic cats and correlated these data with epididymal sperm morphology and the degree of spermatogenic activity. During spring and summer, testes and blood samples were obtained from 37 mixed-breed domestic cats (12 to 48 mo). The epididymal sperm were harvested and evaluated for sperm counts, motility, and morphology. Distal cytoplasmic droplets were not considered a defect, and samples were considered normozoospermic if they contained more than 60% normal sperm (N = 25) or teratozoospermic if they contained less than 45% normal sperm (N = 12). The testicular and serum concentrations of testosterone (T) and 17β-estradiol (E2) were determined with an enzyme immunoassay. The gonadosomatic index and epididymal sperm numbers and motility did not differ between groups. The percentage of normal sperm was higher in normozoospermic (74.3 ± 2.0, mean ± SEM) than in teratozoospermic samples (43.1 ± 1.4). The most prevalent sperm defects in the teratozoospermic group were abnormal acrosomes (9.7 ± 2.0) and bent midpieces (12.2 ± 2.0) or tails (24.0 ± 2.7) with cytoplasmic droplets. Histomorphometric data were similar between groups, although there was a lower Leydig cell nuclear volume in teratozoospermic samples. Normozoospermic samples contained a higher percentage of haploid cells and had a higher index of total spermatogenic transformation than teratozoospermic samples. Serum concentrations of T (0.5 ± 0.1 vs. 0.8 ± 0.4 ng/mL) and E2 (9.5 ± 1.2 vs. 11.4 ± 2.3 pg/mL) and testicular T concentrations (471.6 ± 65.3 vs. 313.4 ± 57.6 ng/g) were similar between groups. However, compared with normozoospermic samples, teratozoospermic samples had higher testicular E2 concentrations (8.5 ± 3.6 vs. 5.4 ± 0.5 ng/g) and a lower T:E2 ratio (31.8 ± 4.1 vs. 87.2 ± 11.6). There were significant correlations between testicular E2 values and percentages of normal sperm ($r = -0.55$) as well as those with primary sperm defects ($r = 0.58$) or abnormal acrosomes ($r = 0.64$). The T:E2 ratio was also correlated with meiotic index ($r = 0.45$) and percentage of normal sperm ($r = 0.58$). In conclusion, a high testicular E2 concentration and a reduced T:E2 ratio were significantly associated with higher ratios of abnormal sperm types, suggesting that the balance between androgens and estrogens is an important endocrine component in the genesis of teratozoospermia in felids.
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Keywords: Androgen; Estrogen balance; Teratozoospermia; Spermatogenesis; Epididymal sperm; Domestic cat

1. Introduction

Spermatogenesis is a cyclical, complex, and continuous process, organized into well-defined seminiferous epithelium cycles, during which spermatogonia proliferate

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0093-691X/$ – see front matter © 2012 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.theriogenology.2012.05.018

and generate sperm [1]. This process is determined genetically and is under highly coordinated endocrine control by gonadotropins and steroid hormones, together with numerous local regulatory factors [2,3]. In the testis, Leydig cells are the main source of androgens, leading to high testicular testosterone concentrations, which are essential for initiation and maintenance of spermatogenesis [4]. The effects of androgens on spermatogenesis are mediated indirectly through Sertoli cells [5], are highly specific, and occur mainly in stages VII and VIII of the germinative cycle of mice [6,7].

Leydig cells are the source of testicular estrogens in the adult testis; although the presence of estrogens has been documented in the male gonad for more than 50 years, until recently, estrogens were considered essentially feminine hormones [8,9]. However, growing evidence has demonstrated that estrogens play a crucial role in regulation of spermatogenesis [3,10,11], acting through nuclear receptors in the testis, as well as in other organs in the male reproductive tract in several species [12–17], including the domestic cat [18,19].

Several studies have attempted to explain the role of estrogens in testicular function [20]. Estrogens exert some control on testosterone biosynthesis in Leydig cells through direct inhibitory effects in the testis [21,22]. Furthermore, a direct antiapoptotic effect was observed in the germinative cells of the seminiferous epithelium of mice with high testicular concentrations of 17β-estradiol [23]. In addition to direct effects on the testis, estrogens influence sperm concentrations and the acquisition of a number of the motile, morphologic, and biochemical properties of sperm, by regulating fluid absorption in the efferent ducts and epididymis [15,24,25]. A significant reduction in the percentage of mobile sperm occurred in epididymal sperm from estrogen receptor-deficient mice (knockout ERα) [26], as well as more severe defects in flagella, such as strongly coiled tails [27]. In contrast, there were high estradiol concentrations in seminal plasma in men with oligozoospermia or oligoteratozoospermia [28,29].

In cats, it is common to detect more than 60% morphologically abnormal sperm in the ejaculate. This teratozoospermic condition has reproductive relevance, because all 28 felid species, with the exception of the domestic cat, are threatened by extinction to some extent [30,31]. The causes and mechanisms involved in teratozoospermia are not fully understood; however, genetic, environmental, and hormonal factors, such as low testosterone concentrations, are likely involved [32,33]. Teratozoospermia occurs periodically in domestic cats and is characterized by increased production of abnormal sperm because of factors such as seasonal regulation, sexual abstinence, or nutrition and health status [34,35]. This sporadic teratozoospermia is usually accompanied by a low sperm count, in contrast to the increased sperm production observed in several other persistently teratozoospermic animals [36,37]. Studies in wild and domestic cats indicate a number of possible factors involved in the etiology and mechanisms of teratozoospermia in felids [30,35]; however, the relationship between the testicular endocrine balance and teratozoospermia is unknown. In this study, we investigated the testicular androgen:estrogen balance in domestic cats by measuring serum and testicular concentrations of testosterone (T) and 17β-estradiol (E2) and correlated these data with epididymal sperm morphology and the degree of spermatogenic activity. We hypothesize that the testicular balance between T and E2 has a role in mechanisms underlying felid teratozoospermia.

2. Materials and methods

2.1. Chemicals

Unless stated otherwise, all chemicals were of reagent grade and were obtained from Sigma, Chemical Co. (St. Louis, MO, USA).

2.2. Animals and orchiectomy

A total of 37 mixed-breed domestic cats (*Felis catus*) aged between 12 and 48 mo (20.0 $\pm$ 1.5) were used in this study. The animals were obtained from private owners and were clinically healthy at examination. All animals were subjected to bilateral orchiectomy. To avoid seasonal effects on testicular function, samples were collected only during the spring and summer, which are considered the most favorable seasons for reproduction in cats. After 12 h of fasting, the animals were anesthetized with a combination of ketamine hydrochloride (Vetaset, Fort Dodge, São Paulo, SP, Brazil, 20 mg/kg im) and xylazine (Rompun 2%, Bayer, São Paulo, SP, Brazil, 1.0 mg/kg im), and the surgical procedure was performed according to standard techniques for cats. Shortly after the animals were anesthetized, a blood sample was collected, and the serum was separated and frozen at -20 °C pending subsequent hormonal analyses. Testis length and width was measured with calipers and testicular volume [36] used to calculate the gonadosomatic index, which is the expression of the mass of the testes as a percentage of the total body mass (testis wt/body wt $\times$ 100). The Ethics

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