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Blood and seminal plasma concentrations of selenium, zinc and testosterone and their relationship to sperm quality and testicular biometry in domestic cats

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ABSTRACT

The aim of this study was to assess seminal plasma (SP) and serum concentrations of zinc (Zn), selenium (Se) and testosterone (T) in domestic cats and determine whether these are related to sperm quality and testicular biometry. Six tomcats were collected using an artificial vagina and sperm analysis included motility by CASA, morphology, plasma membrane integrity, and sperm count. Serum and SP were submitted to total T concentration determination using a solid-phase radioimmunoassay technique while Zn and Se were measured by atomic absorption spectroscopy. Serum T concentrations were greater compared to SP concentrations, but both values were significantly correlated. Se concentrations were higher in serum, whereas SP had greater Zn values. Concentrations of Se, Zn and T were not correlated with each other either in serum or SP. Negative correlations were detected between Se concentrations in SP and total sperm head defects, and between Se concentrations in serum and VAP, VSL, STR, and LIN. Serum concentrations of Zn were negatively correlated with total abnormal sperm and midpiece defects and positively related to progressive motility. Both serum and SP concentrations of T had no relationship with sperm quality. Concentrations of Se exhibited a negative correlation with total testicular weight, whereas T concentrations in SP and serum were correlated with total testicular volume and weight. In conclusion, both Se and Zn concentrations in serum were correlated to sperm quality variables in the domestic cat, thus, making these potential candidates for fertility markers.

1. Introduction

Seminal plasma (SP) originates from different regions of the male genital tract (rete testis, epididymis, and accessory sex glands) and has a complex composition which includes ions, organic and nitrogenous substances, among other compounds (Mann and Lutwak-Mann, 1981). Aside from its function in sperm transport and survival in the female reproductive tract (Troedsson et al., 2005), this non-cellular

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component of semen has also been implicated in sperm maturation, metabolism and function, thus, affecting morphology, motility and the fertilizing ability of spermatozoa (Bleau et al., 1984; Carpino et al., 1998; Colagar et al., 2009; Henkel et al., 1999; Mann and Lutwak-Mann, 1981; Massányi et al., 2004; Safarinejad and Safarinejad, 2009).

Zinc (Zn) and selenium (Se) are trace elements known to be important for normal reproductive function in males. Both elements have been involved in testicular development and spermatogenesis (Behne et al., 1996; Marin-Guzman et al., 2000a; Merker and Günther, 1997; Yamaguchi et al., 2009). In addition, Zn has been related to epididymal sperm maturation (Henkel et al., 2003), stabilization of sperm membrane and nuclear chromatin (Björndahl and Kvist, 2010; Kendall et al., 2000), antioxidant and antibacterial functions (Edstrom et al., 2008; Gavella et al., 1999), and motility modulation (Henkel et al., 1999, 2003). Se exerts its effects on male reproductive physiology mainly through two selenoproteins, phospholipid hydroperoxide glutathione peroxidase (GPx4), located at the midpiece (mGPx4; enzymatic and structural functions) and nucleus (nGPx4; stabilization of condensed chromatin) of spermatozoa, and selenoprotein P which is essential for the maintenance of adequate concentrations of Se (Conrad et al., 2005; Olson et al., 2005; Ursini et al., 1999). Se may also act as a component of glutathione peroxidases (GPx) in SP, therefore, assisting in the protection of spermatozoa against oxygen radicals-induced damages (Kantola et al., 1988). Nevertheless, despite the fact that Zn and Se are undoubtedly necessary for sperm function, whether their concentrations are suitable biomarkers of male fertility remains controversial (Bleau et al., 1984; Chia et al., 2000; Colagar et al., 2009; Roy et al., 1990; Safarinejad and Safarinejad, 2009; Wong et al., 2001).

Testosterone (T) is also considered essential for both spermatogenesis and epididymal maturation of spermatozoa (Robaire and Hamzeh, 2011; Sofikitis et al., 2008). Human subjects defined as infertile have lesser T concentrations in serum and SP when compared to fertile counterparts (Luboshitzky et al., 2002; Zhang et al., 2010). Likewise, a potential association between low T concentrations and teratospermia has been reported in cats (Howard et al., 1990). Additionally, male cats exhibiting seasonal variations in T concentrations have also demonstrated fluctuations in sperm traits (Blottner and Jewgenow, 2007; Tsutsui et al., 2009). Thus, T concentration seems to be a good indicator of sperm quality in this species.

Poor nutrition in felid species has proven to be detrimental to sperm quality (Howard and Allen, 2007; Paz et al., 2006). However, despite the available information in livestock and laboratory animals, there are no data addressing Se and Zn concentrations and the relation to sperm quality in felines. This lack of information includes the domestic cat, which is considered an important research model for wild felines. Therefore, the objective of this study was to determine the concentrations of Se, Zn, and T in blood serum and SP of domestic cats and investigate whether these values are related to testicular biometry and sperm count, morphology, motion parameters, and plasma membrane integrity (PMI).

2. Material and methods

2.1. Animals

Six adult mixed-breed male cats, 4–7 years old, were used as semen donors. All animals were housed at the Veterinary School research cattery for at least 1 year prior to semen collection. Thereby, cats were maintained at similar environmental, social, and nutritional conditions. Animals were exposed to natural lighting (>12 h of light/day; 22°53′09″ S; 48°26′42″ W), fed with dry commercial cat food (FIT 32, Royal Canin) and water *ad libitum*.

2.2. Semen and blood collection

Semen samples were collected during the breeding season (October to December) using an artificial vagina and with at least a 2-days interval between collections. All cats were submitted to semen collection on the same day. A total of three ejaculates from each cat ($n = 18$ ejaculates) were used to assess sperm motility, morphology and PMI. For sperm count determination and SP recovery, each tomcat was collected on another 16 occasions ($n = 96$ ejaculates).

Two blood samples were collected from each cat (November and December) by venipuncture and under manual restraint. All males were collected on the same day, and first and second blood collections were performed between 9:00 and 10:00 am and 5:00 and 6:00 pm, respectively. Blood serum was recovered following centrifugation at $1500 \times g$ for 10 min and then stored at −80 °C until quantifications occurred.

2.3. Semen analysis

Immediately after collection, semen was diluted with 100 μL HEPES-buffered Ham's F-10 0.6% BSA medium. An aliquot was placed on a preheated (38 °C) Makler counting chamber and sperm motion parameters were assessed by computer-assisted sperm analysis (CASA) (HTM, IVOS 12; Hamilton Thorne Research, Beverly, MA) using the domestic cat setup (Villaverde et al., 2009). At least three fields per sample were scanned. Morphological analysis of 200 cells per sample was performed after sperm staining with 1% Fast Green FCF/1% Rose Bengal (Pope et al., 1991). For PMI determination, 10 μL of semen was added to 40 μL of a 3% sodium citrate solution containing propidium iodide (5 μg/mL; Sigma–Aldrich Co.) and carboxyfluorescein diacetate (9.2 μg/mL; Sigma–Aldrich Co.). After a 10-min incubation period in the dark, 200 cells were assessed using an epifluorescence microscope. Live spermatozoa stained green while dead cells stained red or exhibited dual staining (red and green).

2.4. Sperm count and measurement of selenium, zinc and testosterone

To increase SP retrieval, semen was collected twice consecutively with a 5–10-min interval between collections. Samples from the same male were pooled (first and second ejaculates) and centrifuged for 5 min at $700 \times g$. Sperm