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Expression of anti- and pro-apoptotic genes in testicular tissue of domestic cat

Sylwia Prochowska, Agnieszka Partyka, Wojciech Niżański
Reproduction Department with Farm Animal Clinic, Wroclaw University of Environmental and Life Sciences, Poland.
sylwia.prochowska@up.wroc.pl

Teratospermia is a common phenomenon in felids. Reduced germ cell apoptosis is one of the mechanisms proposed to explain its occurrence. Programmed cell death plays an important role in the elimination of abnormal sperm cells, therefore disturbances in germ cell depletion during spermatogenesis may result in higher presence of malformed sperm cells in the ejaculate. So far this hypothesis was not confirmed by measuring the expression of genes involved in the apoptotic process. Also, in many seasonally breeding species apoptotic processes were more pronounced during testicular regression in non-reproductive season. For male cat data about seasonality are contradictory. Although tomcats do not show typical testicular regression and they produce sperm all the year, several authors reported decreased semen quality during non-breeding season. For cats TUNEL labeling showed no influence of season on apoptosis in testicular tissue, but no studies on the level of gene expression exist. The aim of this study was to assess if the expression of pro- and anti-apoptotic genes in testicular tissue in domestic cats differ: 1) between normospermic and teratospermic donors and 2) between reproductive and non-reproductive season.

The study samples consisted of testicular tissue obtained from 12 mixed-breed tomcats after a routine castration. Urethral semen was collected before the castration and morphology of semen was assessed after eosin-nigrosin staining. Five cats were classified as normospermic (>60% normal spermatozoa in semen sample) and 7 cats as teratospermic (<60% normal spermatozoa in semen sample). Additionally, the cats were divided in two groups: orchiectomized during reproductive season (March-September, n=6) and orchiectomized during non-reproductive season (December-February, n=6). Samples (100 mg of tissue from each testis) were kept in RNAlater® solution in 5°C for 24 hours, then snap frozen and stored at -80°C until evaluation. RNA was extracted and gene expression was examined by RT-qPCR. House keeping genes were GAPDH and RPS7; anti-apoptotic genes were BCL2 and BCL2L1, and pro-apoptotic genes were BAX and BAD. Relative expression was calculated by Pfaffl method. Data are given as median values ±SD. Statistical analysis was performed by Student t-test. All examined genes were expressed in feline testicular tissue. Teratospermic donors showed the tendency for higher expression of both anti-apoptotic genes (more pronounced for BLC2), but the difference was not statistically significant (p<0.05). Comparison of samples collected during reproductive and non-reproductive season revealed significantly higher expression of both anti-apoptotic genes (BCL2L1 and BLC2) during non-reproductive season (p>0.05). For pro-apoptotic genes (BAX and BAD), no significant differences between groups were found (p<0.05). The results of this study did not support the hypothesis that teratospermia is directly and certainly connected with reduced germ cells apoptosis. As there may be several different causes of the presence of malformed sperm cells in the semen, more studies on bigger group of consistently teratospermic cats are required. We found higher expression of anti-apoptotic genes during non-reproductive season. We hypothesize that a compensatory defense mechanism, similar to that proposed for teratospermia, may plays a role (decreased level of apoptosis to increase the sperm output at the expense of semen quality). This hypothesis may explain lower quality of semen during non-breeding season reported by other authors.