UNILATERAL TESTICULAR DEGENERATION IN DOGS ADVERSELY AFFECTS SPERMATOZOA CHARACTERISTICS IN THE TESTIS AND EPIDIDYMIS

[1*]Chike F. Oguejiofor, [1]Kenneth O. Anya, [2]Clifford N. Abiaezute, [1]Nnaemeka K. Ogbanya
[1]Department of Veterinary Obstetrics and Reproductive Diseases
[2]Department of Veterinary Anatomy
Faculty of Veterinary Medicine, University of Nigeria, Nsukka
**Corresponding author: E-mail: chike.oguejiofor@unn.edu.ng; Phone: +2348038993103*

ABSTRACT
Testicular degeneration (TD) is associated with poor fertility in affected dogs. The study investigated the specific effects of unilateral testicular degeneration (UTD) on spermatozoal parameters in the testes and epididymides of dogs. Ten sexually mature (7-12 months old) Nigerian indigenous breed of dogs comprising 5 normal dogs (N; both testes normal and present in the scrotum) and 5 dogs with UTD (one normal scrotal testis and one degenerated scrotal testis) were used for the study.The testes and epididymides were removed via orchidectomy and sperm analyses (total and progressive motility, vitality, total abnormalities and concentration) were performed. The samples were grouped as N1 (right normal testis); N2 (left normal testis); UTD-N (normal testis in dogs with UTD); UTD-D (degenerated testis in dogs with UTD). Data were analysed using one-way ANOVA. The UTD-D group had significantly lower ($p<0.001$) epididymal sperm total and progressive motility, vitality and concentration, higher total sperm abnormalities ($p<0.001$) and lower testicular sperm concentration ($p<0.01$) compared to the contralateral UTD-N and the N testes. The UTD-N group also had significantly lower epididymal sperm total motility ($p<0.01$) and progressive motility ($p < 0.05$), and higher total abnormalities ($p<0.05$) compared to the N groups. Our study demonstrated that UTD in dogs adversely affected spermatozoa in the affected testis and epididymis. There was also evidence of compromised spermatozoa in the testis and epididymis contralateral to the degenerated testis. Taken together, these sperm abnormalities are likely to have adverse effect on the fertility of affected dogs.
Keywords: testicular degeneration, sperm characteristics, spermiogram, canine

INTRODUCTION
Dogs are mainly bred for companionship, security and hunting, and to provide valuable source of income for breeders. In addition, the slaughter and consumption of dog meat is popular in many countries worldwide including Nigeria (Podberscek, 2009; Ehimiyein *et al*., 2014). Therefore, infertility is likely to cause significant economic losses in canine breeding.

The testis is the primary organ of reproduction in male animals and functions in spermatogenesis (sperm production) and steroidogenesis (testosterone production). Testicular degeneration (TD) is considered as one of the most common causes of poor semen quality and acquired infertility in male dogs (Fontbonne, 2011; Domingos and Salomão, 2011) with a prevalence of 15-58% in dogs (Ortega-Pacheco *et al.*, 2006; Câmara *et al*., 2014). TD involves deterioration in the structure of the testis with a consequent loss of testicular function (Turner, 2007). It may affect one (unilateral) or both (bilateral) testes, involving parts of the testis or the whole testis. Acute TD can result secondary to a known insult such as exposure of the testes to toxins, radiation, extreme scrotal temperature, scrotal trauma, autoimmune disease, certain nutritional deficiencies, and infection with pathogenic organisms (Turner, 2007; Obi *et al*., 2013). Idiopathic TD has no identifiable underlying cause and has also been reported in dogs (Rehm, 2000; Fontbonne, 2011).

Spermatozoa can be recovered from the cauda epididymides of dogs by percutaneous epididymal aspiration, following castration or at post mortem, for use in the investigation of sperm quality and spermatozoal characteristics (Varesi *et al*, 2013; Chima *et al*., 2017; Bhanmeechao *et al*., 2018) and for artificial breeding of bitches (Wydooghe *et al*., 2016). Ejaculated semen comprises sperm from both testes and also secretions of the accessory sex glands. Thus while evaluation of the ejaculate is used to assess semen for fertility potential, it precludes the investigation of specific effects of testicular disorders and their impact on spermatozoa. Moreover, there is little available information on the effect of TD on epididymal or testicular spermatozoa in dogs. Therefore, the aim of the study was to investigate the specific effects of unilateral testicular degeneration (UTD) on spermatozoal parameters in the testes and epididymides of affected dogs. The specific objectives were to evaluate the effects of UTD on epididymal sperm motility, vitality, morphological abnormalities and concentration, and testicular sperm concentration.

MATERIALS AND METHODS

Animals

Ten sexually mature (7-12 months old) apparently healthy Nigerian indigenous breed of dogs comprising 5 normal dogs (N; both testes normal and present in the scrotum) and 5 dogs with UTD (one normal scrotal testis and one degenerated scrotal testis) were used for the study. Only dogs with a history of progressive unilateral scrotal testicular atrophy were selected and UTD was confirmed retrospectively following testicular histopathology. Animal welfare was observed in accordance with the National Research Council's Guide for the Care and Use of Laboratory Animals (2011) and the protocol was approved by the Research Ethics Committee of the Faculty of Veterinary Medicine (2016/10-173537). Orchidectomy (open castration) was performed following standard surgical procedure as previously described (Hassan and Hassan, 2003) and the testes and epididymides were dissected, and then used for sperm analyses.

Epididymal and testicular sperm analyses

Sperm evaluation was performed as previously described (Seed *et al*., 1996; Cooper, 2010). Sperm motility was determined using the sperm diffusion in phosphate buffered saline (PBS; pH 7.4, 37 °C) from sectioned cauda epididymis. Sperm motility (%) was determined at ×400 using a phase-contrast microscope (Motic B3; Motic, Carlsbad, CA, USA) equipped with a stage slide warmer (TCS-100; Amscope, Ivrine, CA, USA) set at 37 °C. Sperm vitality (%) was evaluated using eosin-nigrosin vital staining method. Sperm morphological abnormalities were evaluated using eosin-nigrosin staining and phase-contrast microscopy. All values in percentage were determined by examining 200 sperm cells in duplicates. Cauda epididymal and testicular sperm concentrations were determined following cauda epididymal/testicular tissue homogenization in PBS and sperm cells were counted using a haemocytometer (Weber, England). Total sperm count was expressed as number of sperm per gram cauda epididymis and per gram testis, respectively.

Data analysis

Data were analyzed using One-way analysis of variance (ANOVA) built in SPSS, version 20 (IBM Corp, Armonk, NY, USA). Significant differences between means were confirmed using Tukey's honestly significant difference post hoc test. Results were considered significant when $p < 0.05$.

RESULTS

The results of the sperm analysis in normal dogs and dogs with UTD are shown in Table 1. There were no significant differences ($p > 0.05$) in all the spermiogram parameters between the two normal testes (N1 and N2) in the normal dogs. Both N groups also did not differ from the normal testes (UTD-N) in dogs with UTD with respect to epididymal sperm vitality and concentration and testicular sperm concentration. However, UTD-N group had significantly lower epididymal sperm total motility ($p < 0.01$) and progressive motility ($p < 0.05$) and higher total abnormalities ($p < 0.05$) compared to the N groups. The predominant sperm abnormalities observed included presence of cytoplasmic droplets, bent tails and coiled tails. On the other hand, there were significant differences in all the spermiogram parameters in the degenerated testes (UTD-D) when compared to the contralateral normal testes (UTD-N) in dogs with UTD and also when compared to the two normal testes (N1 and N2) in the normal dogs. The UTD-D group had significantly lower epididymal sperm total and progressive motility, vitality and concentration ($p < 0.001$) and higher total abnormalities ($p < 0.001$) compared to the contralateral UTD-N and the N testes. The predominant sperm abnormalities observed included presence of detached head, bent midpiece, coiled midpiece (dag defect) and coiled tails. Moreover, testicular sperm concentration was significantly decreased in the UTD-D testes when compared to the contralateral UTD-N testes ($p < 0.01$) and the N testes in normal dogs ($p < 0.001$).

Table 1. Epididymal and testicular spermatozoal parameters in dogs with normal testes and unilateral testicular degeneration

Sperm parameters	N1	N2	UTD-N	UTD-D
Epididymal sperm total motility (%)	83.6 ± 2.7^a	84.8 ± 3.0^a	71.2 ± 5.8^b	8.4 ± 5.1^c
Epididymal sperm progressive motility (%)	74.4 ± 4.7^a	71.2 ± 5.0^a	60.2 ± 7.8^b	3.6 ± 4.2^c
Epididymal sperm vitality (%)	94.6 ± 2.3^a	94.2 ± 2.8^a	87.6 ± 4.8^a	27.2 ± 8.3^b
Epididymal sperm total abnormalities (%)	28.0 ± 2.7^a	27.2 ± 3.1^a	39.0 ± 3.2^b	66.4 ± 8.7^c
Epididymal sperm concentration ($x10^6$/g epididymis)	926.0 ± 23.9^a	912.4 ± 19.1^a	874.0 ± 78.7^a	3.29 ± 2.1^b
Testicular sperm concentration ($x10^6$/g testis)	31.6 ± 5.7^a	29.3 ± 4.8^a	23.2 ± 9.3^a	5.81 ± 2.9^b

N1 (right normal testis); N2 (left normal testis); UTD-N (normal testis in dogs with UTD); UTD-D (degenerated testis in dogs with UTD). Values represent mean ± SD. Rows with different superscripts indicate significant differences ($p < 0.05$).

DISCUSSION

The study provided evidence that UTD adversely affected spermatozoa parameters in the testes and epididymides of dogs. UTD decreased epididymal sperm total and progressive motility. A low % of progressively motile sperm has also been reported in the semen of stallions with TD (Blanchard *et al*., 2000). Decreased sperm motility may be related to abnormal sperm formation and the observed increase in dead sperm and sperm with morphologic abnormalities. There was also increased % of dead epididymal sperm (decreased sperm vitality) in dogs with UTD. This may be related to a disruption of the normal processes in spermatogenesis and sperm epididymal maturation consequent upon the testicular insult. A significantly higher percentage of morphologically abnormal sperm was observed herein in the dogs with UTD. Câmara *et al*. (2014) similarly reported high sperm abnormality in dogs with severe TD. Interestingly, our study observed a higher % of sperm total abnormalities in dogs with UTD compared to the previous study. This may be due to differences in the source of sperm sample. Herein, sperm was harvested from the cauda epididymis whereas the previous study utilized sperm from the vas deferens. In addition, a higher % of detached head, bent midpiece and dag defect were observed in the UTD-D group, which are known to have significant negative effect on fertility (Kolster, 2018).

In this study, TD also decreased the testicular and epididymal sperm concentration. This may be a consequence of atrophy of the testicular parenchyma, degeneration of seminiferous tubules and decreased spermatogenesis (spermatogenic arrest). Since the cauda epididymis is site of sperm storage, the lowered epididymal sperm concentration may reflect a decrease in sperm production in the testis. Similarly, there was lowered daily sperm production per milliliter of testis and decreased sperm concentration in stallions with TD (Blanchard *et al*., 2001). Azoospermia has also been reported in the semen and vas deferens fluid of dogs with TD (Fontbonne, 2011; Câmara *et al*., 2014). Moreover, the findings suggest that TD in one testis can impair spermatozoa in the contralateral testis and epididymis. However, the mechanism for this effect (whether direct or indirect) was unclear due to the unknown underlying aetiology of UTD in the affected dogs. Some spermatozoal parameters (vitality and concentration) were not significantly different between the testes in normal dogs and the contralateral testes in UTD dogs. However, it is likely that the overall fertility outcome in dogs with UTD will depend on the progression and severity of TD. Taken together, lowered epididymal sperm motility, vitality and concentration, increased epididymal sperm abnormalities, and decreased testicular sperm concentration can have adverse effect on the fertility of affected dogs (Johnston *et al*., 2001; Robert *et al*., 2016; Kolster, 2018).

In summary, our study demonstrated that UTD in dogs adversely affected spermatozoa in the testis and epididymis and also showed evidence of compromised spermatozoa in the contralateral testis and epididymis. Our findings provide more information on alterations in sperm quality in relation to UTD. Although sperm cells may be recovered from the cauda epididymides of affected dogs for application in assisted reproductive technologies, further studies could investigate the functional capacity and fertilizing potential of such recovered spermatozoa.

REFERENCES

Podberscek, A.L. 2009. Good to pet and eat: the keeping and consuming of dogs and cats in South Korea. *J Soc. Issues*. 65: 615-632.

Bhanmeechao, C., Srisuwatanasagul, S., Prapaiwan, N., Ponglowhapan, S. 2018. Reproductive aging in male dogs: the epididymal sperm defects and expression of androgen receptor in reproductive tissues. *Theriogenology.*108: 74-80.

Blanchard, T., Johnson, L., Roser, A.J. 2000. Increased germ cell loss rates and poor semen quality in stallions with idiopathic testicular degeneration. *J Equine Vet. Sci.* 20: 263-265.

Blanchard, T.L., Johnson, L., Varner, D.D., Rigby, S.L., Brinsko, S.P., Love, C.C., Miller, C. 2001. Low daily sperm output per ml of testis as a diagnostic criteria for testicular degeneration in stallions. *J Equine Vet. Sci*. 21: 11, 33-35.

Câmara, L.B.R.M., Câmara, D.R., Maiorino, F.C., Silva Júnior, V.A., Guerra, M.M.P. 2014. Canine testicular disorders and their influence on sperm morphology. *Anim. Reprod*. 11: 32-36

Chima, U., Abu, A., Dawuda, P., Kisani, A., Ahemen, T. 2017. Effect of storage time on cauda epididymal sperm parameters of Nigerian local dogs. *Open J Vet. Med*. **7:** 151-161.

Cooper, T.G. Editor. WHO laboratory manual for the examination and processing of human semen. 5th edn. World Health Organization Geneva, Switzerland: WHO Press; 2010.

Domingos, T.C.S., Salomão, M.C. 2011. Diagnostic tools of testicular disorders in dogs: review. *Rev. Bras. Reprod. Anim*. 35: 393-399.

Ehimiyein, A., Audu, S., Ehimiyein, I. 2014. The role of dog trading and slaughter for meat in rabies epidemiology with special reference to Nigeria- a review. *J Exp. Biol. Agric. Sci*. 2: 130-136.

Fontbonne, A. 2011. Infertility in male dogs: recent advances. *Rev. Bras. Reprod. Anim*. 35: 266-273.

Hassan, A.Z., Hassan, F.B. 2003. An introduction to veterinary practice. Zaria, Nigeria: Ahmadu Bello University Press Ltd. p. 288.

Johnston, S.D., Kustritz, M.V.R., Olson, P.N.S. Semen collection, evaluation, and preservation. In: Johnston SD, Kustritz MVR, Olson PNS, editors.Canine and FelineTheriogenology, 1st edn. Philadelphia: W.B. Saunders; 2001. p. 287-306.

Kolster, K.A. 2018. Evaluation of Canine Sperm and Management of Semen Disorders.*Vet Clin Small Anim*. 48: 533-545.

National Research Council: Institute for Laboratory Animal Research. The guide for the care and use of laboratory animals. 8th ed. Washington DC: The National Academies Press; 2011.

Obi, C.F., Obidike, R.I., Ezeh, I.O., Omoja, V.U., Iheagwam, C.N., Idika, I.K., Ezeokonkwo, R.C. 2013. Effects of Trypanosoma brucei infection and diminazene aceturate therapy on testicular morphology and function of Nigerian local dogs. *Vet. Parasitol*. 196: 283-288.

Ortega-Pacheco, A., Rodríguez-Buenfil, J.C., Segura-Correa, J.C., Bolio-Gonzalez, M.E., Jiménez-Coello, M., Linde Fosberg, C. 2006. Pathological conditions of the reproductive organs of male stray dogs in the tropics: prevalence, risk factors, morphological findings and testosterone concentration. *Reprod. Domest. Anim.* 41: 429-437.

Rehm, S. 2000. Spontaneous testicular lesions in purpose-bred Beagle dogs. *Toxicol. Pathol*. 28: 782-787.

Robert, M.A., Jayaprakash, G., Pawshe, M., Tamilmani, T., Sathiyabarathi, M. 2016. Collection and evaluation of canine semen- a review. *Int. J Sci. Environ. Tech*. 5: 1586-95.

Seed, J., Chapin, R.E., Clegg, E.D., Dostal, L.A., Foote, R.H., Hurtt, M.E. 1996. Methods for assessing sperm motility, morphology, and counts in the rat, rabbit, and dog: A consensus report. *Reprod Toxicol*. 10: 237-44.

Turner, R.M.O. 2007. Pathogenesis, diagnosis, and management of testicular degeneration in stallions. *Clin. Tech. Equine Pract*. 6: 278-284.

Varesi, S., Vernocchi, V., Faustini, M., Luvoni, G.C. 2013. Quality of canine spermatozoa retrieved by percutaneous epididymal sperm aspiration. *J Small Anim. Pract*. 54: 87-91.

Wydooghe, E., Snoeck, F., Van Soom, A. 2016. Establishment of live birth following intravaginal artificial insemination with chilled epididymal dog semen collected post mortem: case report. Proceedings of the International Symposium on Canine and Feline Reproduction (ISCFR), June 2016, Paris, France. p. 231.