UNIVERSITI PUTRA MALAYSIA

COMPARISON OF TESTICULAR CELL POPULATION INDEXES IN NORMAL DOGS USING FINE NEEDLE ASPIRATION AND IMPRINT TECHNIQUES

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By

ARASH KAMALI

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfillment of the Requirements for the Degree of Master of Veterinary Science

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DEDICATION

I would like to dedicate this work to my beloved wife, Mahfam and my sweet daughter, Parmys who are the reasons for every heartbeat of mine and my purpose to live life for another day

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the degree of Master of Veterinary Science

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Chairperson: Associate Professor Gurmeet Kaur Dhaliwal, PhD
Faculty : Veterinary Medicine

Testicular cell population indexes have been described in human and a few domestic species of animals to evaluate spermatogenesis. Fine needle aspiration (FNA) and imprints are different techniques available to obtain specimens for cytological evaluation of the testicle. FNA is the less invasive method compared to the imprint. This study was conducted to determine and compare testicular cell population indexes using FNA and imprint methods among dogs of different age groups.

A total number of 35 mixed breed dogs, with no testicular abnormalities were categorized into four different age groups; junior (0.5 - <2 years; N=7), adult (2 - <4.5 years; N=10), mature (4.5 - <7 years; N=11) and senior (7 - <10 years; N=7). Three FNA samples and two imprints were obtained from different but consistent locations of each testicle. Cytological evaluation was performed after staining with Wright's stain and based on a random count of 500 cells from each slide.

The results of this study demonstrated no significant differences between the three different FNA locations or the two imprint areas. There were also no significant differences in the testicular cell population indexes between the left and right testicles in either of the techniques. Therefore, regardless of the sampling site, the same reference range of testicular cell population indexes can be used for interpretation.

The mean of the testicular cell population indexes for FNA versus imprint samples are as follow: sperm index (SI): 40.54 vs. 23.11, Sertoli cell index (SEI): 8.83 vs. 5.11, Sperm-Sertoli cell index (SSEI): 7.20 vs. 6.69, late spermatid index (LSI): 33.55 vs. 27, early spermatid index (ESI): 23.09 vs.42.77 and spermatocyte index (SPI): 2.61 vs.7.08.All testicular cell population indexes were significantly ($P< 0.05$) different between FNA and imprints, except for SSEI. FNA samples consisted of more Sertoli cells and mature spermatogenic cells such as spermatozoa (SI: 40.54 ± 13.36 vs. 23.11 ± 10.88) and late spermatids while imprints contained early spermatids the most. Therefore, for interpretation of the samples, it is highly recommended to choose the reference range of the cell population indexes according to the sampling technique.

Another important factor to be considered is the age of the animal. This is because of the significant differences between testicular cell indexes in different age groups. Based on FNA results, in the junior group, SI and SSEI were significantly lower and SEI and ESI were significantly higher than the mature group. However, the differences of the testicular cell indexes were not so remarkable in imprint samples, except for a higher SEI in junior group compared to the other groups.

The results of this study suggest that FNA is a more useful technique compared to imprint, as it is less invasive and more efficient in detection of spermatozoa and Sertoli cells. Beside that, the age of a dog should be taken into consideration, whenever FNA based testicular cell population indexes are to be interpreted.

Abstrak tesis dikemukakan kepada Senat Universiti Putra Malaysia bagi memenuhi keperluan untuk ijazah Sarjana Sains Veterinar.

PERBANDINGAN INDEKS POPULASI SEL TESTIS PADA ANJING NORMAL DENGAN MENGGUNAKAN TEKNIK ASPIRASI JARUM HALUS DAN BEKAS

Oleh

ARASH KAMALI

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Pengerusi: Profesor Madya Gurmeet Kaur Dhaliwal, PhD
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Indeks populasi sel testis telah dihuraikan dalam manusia serta beberapa spesies haiwan domestik.Aspirasi jarum halus dan teknik bekas merupakan teknik berlainan yang sedia ada untuk memperolehi spesimen bagi penilaian sitologi testis.Teknik aspirasi jarum halus adalah kurang invasif jika dibandingkan dengan teknik bekas.Kajian ini direka untukmenentukan dan membandingkan indeks populasi sel testis dengan mengunakan teknik aspirasi jarum halus dan bekas pada kumpulan anjing yang berbeza dari segi umur.

Tiga puluh lima ekor anjing berbaka campur, yang tiada keabnormalan testis dekategorikan kepada empat kumpulan mengikut umur; iaitu remaja (0.5 - <2 tahun; N=7), dewasa (2 - <4.5 tahun; N=10), matang (4.5 - <7 tahun; N=11) dan senior (7 - <10 tahun; N=7). Tiga sampel aspirasi jarum halus dan dua bekas diperolehi daripada setiap testis pada lokasi berlainan tetapi tekal.Penilaian sitologi dilakukan setelah diwarnakan dengan pewarna Wright dan berasaskan hitungan rawak 500 sel dari setiap slaid.

Keputusan kajian ini menunjukkan tiada perbezaan bererti antara ketiga-tiga lokasi aspirasi jarum halus atau dua kawasan bekas.Juga, tiada perbezaan bererti dalam indeks populasi sel testis antara testis kiri dan kanan dalam teknik kedua-dua tersebut.Oleh itu, rujukanjulat indeks populasi sel testis boleh digunakan untuk tafsiran tanpa mengira lokasi sampel.

Purata indeks populasi sel testis bagi aspirasi jarum halus dan bekasmasing-masing adalah seperti berikut: indeks sperma (SI): 40.54 vs.23.11, indeks sel Sertoli (SEI): 8.83 vs. 5.11, indeks sperma-sel Sertoli (SSEI): 7.20 vs. 6.69, indeks spermatid peringkat akhir (LSI): 33.55 vs. 27, indeks spermatid peringkat awal (ESI): 23.09 vs. 42.8 dan indeks spermatosit (SPI): 2.61 vs. 7.08.Terdapat perbezaan bererti ($P<0.05$) pada kesemua indeks populasi sel testis bagi aspirasi jarum halus serta bekas, kecuali SSEI. Lebih banyak sel Sertoli dan sel matang seperti spermatozoa (SI: 40.54 ± 13.36 vs. 23.11 ± 10.88) serta spematid akhir pada sampel jarum aspirasi halus manakala sample bekas kebanya kannya terdiri daripada spermatid peringkat awal.Oleh itu, bagi

pentafsiran sampel,adalah dicadangkan supaya rujukan indeks populasi sel harus dipilih mengikut teknik pensampelan.

Satu faktor penting yang perlu dipertimbangkan adalah usia haiwan. Ini kerana terdapat perbezaan bererti di kalangan indeks sel testis mengikut umur. Berasaskan kepada keputusan aspirasi jarum halus, bagi kumpulan remaja, SI dan SSEI adalah lebih rendah manakala SEI dan ESI adalah lebih tinggi jika dibandingkan dengan kumpulan matang. Namun, perbezaan indeks sel testis tidak seketara dalam sampel bekas, kecuali SEI yang lebih tinggi dalam kumpulan remaja berbanding dengan kumpulan lain.

Keputusan kajian ini mencadangkan bahawa aspirasi jarum halus adalah teknik yang lebih berguna berbanding dengan teknik bekas kerana ianya kurang invasif serta lebih cekap dalam pengesanan spermatozoa dan sel Sertoli. Di samping itu, usia anjing perlu dipertimbangkan apabila indeks populasi sel testis berasaskan aspirasi jarum halus ditafsirkan.

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I certify that an Examination Committee has met on date of viva voce to conduct the final examination of name of student on his degree thesis entitled "Testicular Cell population Indexes in the normal canine testicle using fine needle aspiration and imprint techniques " in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the student be awarded the Master of Veterinary Science in Small Animal Medicine.

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AAHA	American Animal Hospital Association
ART	Assisted reproductive technology
ESI	Early spermatid index
FNA	Fine needle aspiration
FNB	Fine needle biopsy
FSH	Follicle stimulating hormone
LSI	Late spermatid index
SEI	Sertoli cell index
SI	Sperm index
SSEI	Sperm-Sertoli cell index

CHAPTER 1

INTRODUCTION

Male dog infertility can result in many financial losses to the developing dog breeding industry (Memon, 2007). Furthermore, male factors are assumed to be responsible for 40-50% of pregnancy failures (Lopate, 2012). Clinical evaluation of an infertile male dog is a complicated procedure as the diagnostic procedures to evaluate male fertility are commonly limited in small animal practice (Romagnoli *et al.*, 2009).

Clinical evaluation of an infertile male dog may include a step by step procedure starting with history, patient signalment, followed by physical examination of the reproductive organs. The scrotal skin is evaluated for thickening and the tunica of the testicle for fluid accumulation. Testicular consistency, enlargement, local or diffuse firmness or softness of the testicles might be detectable in physical examination (Lopate, 2012).

Assessment of spermatogenesis is definitely the most important step in the diagnosis of infertility. Semen evaluation is the most important step in this regard (Root Kustritz, 2007). There are different terms to describe the abnormalities of the semen. Azoospermia is the complete absence of spermatozoa in the ejaculate (Lopate, 2012). Oligozoospermia is the low number of spermatozoa in the ejaculate. Dogs with total spermatozoa count less than $22x10^6$ sperm per kg body weight in their ejaculate are considered oligozoospermic (Johnson, 2006).

Testicular ultrasound is a well-tolerated and safe imaging modality, which is helpful for measurement of the testicular volume and detection of some structural abnormalities, which is usually visualized as abruption in the normal homogenous appearance of the testicle. These may present as diffuse or focal hypoechoic or hyperechoic areas within the testicle or epididymis. However, the ultrasonographic appearance of most testicular abnormalities is not commonly specific enough to yield a definitive diagnosis. Therefore, in most cases, a definitive diagnosis remains to be made after fine needle aspiration (FNA) cytology or testicular biopsy (Davidson and Baker, 2009).

While the basic steps in reproductive soundness evaluation commonly provide some useful information to reach a tentative diagnosis, they do not reveal the underlying abnormal processes within the testicles. Moreover, a precise diagnosis is essential for choosing the most appropriate treatment option and determining the prognosis of the case. Therefore, in cases of infertility where a diagnosis is required, a more detailed evaluation by obtaining testicular biopsy or cytological evaluation is required (Root Kustritz, 2006). Testicular biopsy is the gold standard method in the diagnosis of testicular abnormalities and infertility(Meinhard *et al.*,1973;Rosenlund *et al.*, 1998;Arïdoğan *et al.*, 2003). Testicular biopsy specimens provide useful information on tubular diameter and structure. While the testicle can undergo various pathological processes such as inflammation, fibrosis, vascular compromise or neoplasia, the outcome of these processes leads to diagnosis of either of the following categories of spermatogenesis on histopathology: normal spermatogenesis, hypospermatogenesis, maturation arrest, “Sertoli cell only syndrome” and germinal aplasia. Another possibility

is mixed patterns with a combination of two or more conditions. For instance, it is possible to find focal areas with normal spermatogenesis, while the rest of the testicle has undergone degenerative and aplastic changes. Therefore, in order to have a good representative sample from a testicle, it is suggested to obtain multiple samples from different areas (Ahamad *et al*., 2010).

As the preparation and processing of the biopsy specimens are time consuming and require special facilities, it is also possible to provide a touch imprint slide for early cytological evaluation by merely touching the biopsied tissue to a glass slide. This is an easy technique to have a quicker insight of the condition of the testicle. Differentiation of testicular cells is easier in cytology samples compared to histopathology. Therefore, this technique can be used as a complementary method to biopsy. Studies in humans have demonstrated that testicular imprints are very well correlated with histopathology results and in many instances, immediate decision for management of the case can be made upon evaluation of the cytology sample alone (Odabas *et al.,* 1997; Düşmez *et al*., 2011, Yildiz-Aktas *et al*., 2011).

The disadvantage of testicular biopsy is that the procedure can lead to irreversible damage to the testicular tissue. Complications such as vascular injury, devascularization, fibrosis and inflammatory changes have been reported after testicular biopsy (Lopate *et al*., 1989, Hunt and Foote, 1997). Since it is considered as an invasive technique, many owners and breeders hesitate to allow an open biopsy for their dogs. Additionally, many clinicians hesitate to take the risk of obtaining testicular biopsies due to the fear of inducing irreversible damage to the testicular tissue (Romagnoli *et al*., 2009).

After the first application of FNA for diagnosis of testicular abnormalities in 1965 (Odabas *et al.,* 1997), many studies on humans and domestic animals have demonstrated that it is a less invasive method and it commonly does not cause any long-term deleterious effects on spermatogenesis (Gouletsou *et al.*, 2010, 2011, 2012). Moreover, several studies demonstrated a strong correlation between testicular cytology and histopathology of biopsy samples both in cases of infertility and other testicular disorders (Ali *et al*.,1991; Odabas *et al*., 1997; Meng *et al*., 2000; Qublan *et al*., 2002; Bettella *et al*., 2005; Mehrotra and Chaurasia, 2008; Jha and Sayami, 2009; Düşmez *et al.*, 2011). In addition, since it is minimally invasive, multiple samples can be obtained from different locations of the same testicle. These multiple samples are more representative of the processes within the testicle compared to a single biopsy sample (Arïdoğan *et al*., 2003; Jha and Sayami, 2009). FNA mapping, which is sampling from more than four different locations on a testicle, is now one of the most reliable techniques to detect patches of active spermatogenesis in assisted human reproduction (Turek *et al*., 1997; Dajani, 2005; Arïdoğan *et al*., 2003; Beliveau and Turek, 2011).

Testicular cytology can also be utilized to assess the efficiency of spermatogenesis. To evaluate this, different cell population indexes are calculated from the cytological assessment and are compared to normal reference values. The three most important indexes are sperm index (SI), Sertoli cell index (SEI) and sperm-Sertoli cell index (SSEI). SI is the number of spermatozoa per 100 spermatogenic cells and reflects the

level of spermiogenesis. SEI is the number of Sertoli cells per 100 spermatogenic cells and reflects the potential of the whole spermatogenic activity of the testicle. SSEI is the number of spermatozoa per Sertoli cell and reflects the efficiency of spermatogenesis (Romagnoli *et al*., 2009).Testicular cell population indexes have been studied in man with normal and abnormal spermatogenesis (Adhikari, 2009; Batra *et al*., 2011). However, they have not been thoroughly evaluated in all domestic animals.

Initial cytologic characteristics of the canine testicle and a few of the cell population indexes have been reported based on imprint samples. The cell population indexes from FNA samples corresponded with imprints, although the values were not reported (Santos *et al*., 2010). However, studies in humans and alpacas demonstrated differences in some cytological characteristics of FNA and imprint samples (Odabas *et al*., 1997; Stelletta *et al*., 2011). Normal cell population indexes from FNA cytology are still lacking in dogs. Due to this lack of reference values for testicular indexes from FNA, veterinarians and researchers have to rely upon human values as a reference for canine testicular samples, which might lead to misinterpretation (Romagnoli *et al*., 2009).

Sperm production has been shown to change by age from the time of puberty. Very young and very old dogs have reduced number of spermatozoa in their semen (Schubert and Seager, 1991; Seager and Schubert, 1996; Freshman, 2002). Moreover, asthenozoospermia and teratozoospermia are more frequently seen in very young and old dogs (Johnson, 2006). The differences of testicular cell population indexes have not been studied by taking age of the animals into consideration.

Considering the lack of sufficient research in this area, the hypothesis is that the sampling technique and the location of sampling in addition to the age of the animal will result in variation of testicular cell population indexes, even in normal dogs. This can have a great impact on the interpretation of the case. Therefore, to achieve a better understanding of canine testicular cytology and testing this hypothesis, the current study was designed with the following objectives to:

1. determine and compare testicular cell population indexes in normal dogs using FNA and imprint methods.
2. compare testicular cell population indexes derived from different locations of the normal testicle in different age groups of dogs.

The main limitation in this study was the absence of reproductive history (such as previous breeding history or semen analysis) for each animal. In addition, the age of the dogs were mainly estimated based on the dentition, which can also be influenced by other factors such as nutrition and traumatic insult.

REFERENCES

Adhikari, R. (2009) Testicular fine needle aspiration cytology in azoospermic males. *Nepal Medical College Journal*.11(2): 88-91.

Ahamad, M.S., Chowdhury, B.O., Zobair, M. (2010) Testicular fine needle aspiration in male infertility: A Review. *Journal of Chittagong Medical College Teachers' Association*.21(1): 62-65.

Ali, I. (1999). The role of fine needle aspiration cytology of the testis in the diagnostic evaluation of infertility. *British Journal of Urology*.84(4): 485-488.

Ali, M.A., Akhta M., Woodhouse, N., Burges, A., Faulkner, C., Huq, M. (1991). Role of testicular fine-needle aspiration biopsy in the evaluation of male infertility: Cytologic and histologic correlation. *Diagnostic Cytopathology*. 7(2): 128-131.

Amin, A., Monabati, A., Tadayon, A., Attaran, S.Y., Kumar, P.V. (2006) Testicular fine needle aspiration in male infertility. *Acta Cytologica*. 50(2): 147–50.

Arïdoğan, I., Bayazit, Y., Yaman, M., Ersöz, C., Doran, Å. (2003). Comparison of fine needle aspiration and open biopsy of testis in sperm retrieval and histopathologic diagnosis. *Andrologia*. 35(2): 121-125.

Balarini, M.K., de Paula, T.Z.A., Matta, S.P., Peixoto, J.V., Guião-Leite, F.L., Rossi Júnior, J.L., Czermak Junior, A.C., Walker, N.J. (2012) Stages and duration of the cycle of the seminiferous epithelium in oncilla (*Leopardus tigrinus*, Schreber, 1775). *Theriogenology*. 77(5): 873-880.

Barth, A.D., Alisio, L., Avilés, M., Arteaga, A.A., Campbell, J.R., Hendrick, S.H. (2008).Fibrotic lesions in the testis of bulls andrelationship to semen quality.*Animal Reproduction Science*.106(3-4): 274-288.

Bartges, J.1., Boynton, B., Vogt, A.H., Krauter, E., Lambrecht, K., Svec, R., Thompson, S. (2012) AAHA canine life stage guidelines. *Journal of the American Animal Hospital Association.* 48(1):1-11.

Batra, V.V., Khadgawat, R., Agarwal, A., Krishnani, N., Mishra, S.K., Mithal, A., Pandey, R. (2011) Correlation of cell counts and indices in testicular FNAC with histology in male infertility. *Acta Cytologica*. 43(4): 617-623.

Beliveau, M. E.,Turek, P. J. (2011). The value of testicular `mapping' in men with non-obstructive azoospermia. *Asian Journal of Andrology*.13(2): 225-230.

Bettella, A., Ferlin, A., Menegazzo, M., Ferigo, M., Tavolini, I., Bassi, P., Foresta, C. (2005) Testicular fine needle aspiration as a diagnostic tool in non-obstructive azoospermia. *Asian Journal of Andrology*. 7(3): 289-294.

Carrillo, J., Soler, M., Lucas, X., Agut, A. (2012). Colour and pulsed Doppler ultrasonographic study of the canine testis. *Reproduction in Domestic Animals*. 47(4): 655-659.

Cerilli, L. A., Kuang, W., Rogers, D. (2010). A practical approach to testicular biopsy interpretation for male infertility. *Archives of Pathology & Laboratory Medicine, 134* (8), 1197-1204.

Craft, I., Tsirigotis, M., Courtauld, E., Farrer-Brown, G. (1997) Testicular needle aspiration as an alternative to biopsy for the assessment of spermatogenesis. *Human Reproduction.* 12(7):1483-1487.

Dahlbom, M., Måkinen, A., Suominen, J. (1997). Testicular fine needle aspiration cytology as a diagnostic tool in dog infertility. *Journal of Small Animal Practice*.38(11): 506-512.

Dajani, Y.F. (2005) Pitfalls in the pathological diagnosis of male infertility.*Current Diagnostic Pathology*. 11: 264–273.

Davidson, A.P., Baker, T.W. (2009). Reproductive ultrasound of the dog and tom. *Topics in Companion Animal Medicine*.24(2): 64-70.

de Barros,J.B., de Paula,T.A., da Matta, S.L., Fonseca, C.C., Leite, F.L., Rossi Jr, J.L., de Oliveira, P.C., da Costa, E.P. (2007). Sertoli cell index and spermatic reserves in adult captive African lions (Panthera leo, Linnaeus, 1758). *Animal Reproduction Science*.102(3-4):350-356.

Dohle, G.R., Elzanaty, S., van Casteren, N. J. (2011). Testicular biopsy: clinical practice and interpretation. *Asian Journal of Andrology*, 14(1): 88-93.

Doxsee, A.L., Yager, J.A., Best, S.J., Foster, R.A. (2006). Extratesticular interstitial and Sertoli cell tumors in previously neutered dogs and cats: a report of 17 cases. *The Canadian Veterinary Journal*. 47(8): 763.

Düşmez, D., Aydin, O., Akbay, E., Cayan, S., Kanik, E.A. (2011). Touch imprint cytology in biopsy of the infertile testis. *Acta Cytologica*. 45(6): 990-994.

Felumlee, A. E., Reichle, J. K., Hecht, S., Penninck, D., Zekas, L., Dietze Yeager, A. Goggin, J. M., Lowry, J. (2012). Use of ultrasound to locate retained testes in dogs and cats. *Veterinary Radiology & Ultrasound*.53(5): 581-585.

Foley, G. L. (2001). Overview of male reproductive pathology.*Toxicologic pathology*.29(1): 49 –63.

França, L.R., Avelar, G.F., Almeida, F.F. (2006). Spermatogenesis and sperm transit through the epididymis in mammals with emphasis on pigs. *Theriogenology*. 63(2): 300–318.

Freshman J.L. (2002) Semen collection and evaluation. *Clinical Techniques in Small Animal Practice*.17(3): 104-107.

Gnemmi, G., Lefebvre, R.C., Buczinski, S. (2009). Ultrasound imaging of the bull reproductive tract: an important field of expertise for veterinarians. *Veterinary Clinics of North America, Food Animal Practice.* 25(3): 767-779.

Gonzalez-Bulnes, A., Pallares, P., Vazquez, M.I. (2010). Ultrasonographic imaging in small ruminant reproduction. *Reproduction in Domestic Animals.* 45(2): 9-20.

Gottschalk–Sabag, S., Glick, T., Weiss, D.B. (1993) Fine needle aspiration of the testis and correlation with testicular open biopsy. *Acta Cytologica*. 37: 67–72.

Gottschalk-Sabag, S., Wiess, D. B., Folb-Zacharow, N., Zukerman, Z. (1995) Is one testicular specimen sufficient for quantitative evaluation of spermatogenesis? *Fertility and Sterility*. 64(2): 399-402.

Gouletsou, P.G., Galatos, A.D., Leontides, L.S. (2008). Comparison between ultrasonographic and caliper measurements of testicular volume in the dog. *Animal Reproduction Science*.108(12): 1-12.

Gouletsou, P., Galatos, A., Leontides, L., Sideri, A. (2010). Impact of fine or large needle aspiration on the dog's testis: in vitro ultrasonographic, bacteriological, gross anatomy and histological assessment. *Theriogenology*. 74(9): 1604-1614.

Gouletsou, P., Galatos, A., Leontides, L., Sideri, A. (2011) Impact of fine or large needle aspiration on canine testes: Clinical, in vivo ultrasonographic and seminological assessment. *Reproduction in Domestic Animals*.46(4): 712-719.

Gouletsou, P.G., Galatos, A.D., Sideri, A.I., Kostoulas, P. (2012). Impact of fine needle aspiration (FNA) and of the number of punctures on the feline testis: Clinical, gross anatomy and histological assessment. *Theriogenology*. 78(1): 172-181.

Gradner, G., Dederichs, D., Hittmair, K. (2006). Intra-abdominal testicular torsion in a cryptorchid dog. *Wiener Tierärzliche Monatsschrift*. 93(3): 58.

Grieco, V., Riccardi, E., Greppi, G., Teruzzi, F., Iermanò, V., Finazzi, M. (2008). Canine testicular tumours: a study on 232 dogs. *Journal of Comparative Pathology*. 138(2): 86-89.

Hamm, B. (1997). Differential diagnosis of scrotal masses by ultrasound. *European Radiology*. 7(5): 668-679.

Han U., Adabağ, A., Köybaşioğlu, F., Onal, B.U. (2006). Clinical value of cell counts and indices in testicular fine needle aspiration cytology in primary infertility: diagnostic performance compared with histology. *Analytical and Quantitative Cytology and Histology / the International Academy of Cytology [and] American Society of Cytology*. 28(6): 331-336.

Heath, A.M., Carson, R.L., Purohit, R.C., Sartin, E.M., Wenzel, J.G., Wolfe, D.F. (2002 a). Effects of testicular biopsy in clinically normal bulls. *Journal of the American Veterinary Medical Association*.220(4): 507-512.

Heath, A.M., Pugh, D., Sartin, E., Navarre, B., Purohit, R. (2002 b). Evaluation of the safety and efficacy of testicular biopsies in llamas. *Theriogenology*.58(6): 1125-1130.

Hecht, S., King, R., Tidwell, A.S., Gorman, S.C. (2004). Ultrasound diagnosis: intra-abdominal torsion of a non-neoplastic testicle in a cryptorchid dog. *Veterinary Radiology & Ultrasound.* 45(1): 58-61.

Hess, R., França, L. (2009). Spermatogenesis and cycle of the seminiferous epithelium.*Advances in Experimental Medicine and Biology.* 636: 1-15.

Hunt, W., Foote, R.H. (1997). Effect of repeated testicular biopsy on testis function and semen quality in dogs. *Journal of Andrology*.18(6): 740-744.

Hussein, A. (2013) Evaluation of diagnostic testis biopsy and the repetition of testicular sperm extraction surgeries in infertility patients. *Fertility and Sterility.* 100(1): 88-93.

Jha, R., Sayami, G. (2009) Testicular fine needle aspiration in evaluation of male infertility. *Journal of the Nepal Medical Association*.48(173): 78-84.

Johnsen, S.G. (1970) Testicular biopsy score count-a method for registration of spermatogenesis in human testes: normal values and results in 335 hypogonadal males. *Hormones.* 1(1): 2-25.

Johnson, C. (2006). Current concepts on infertility in the dog. *Waltham Focus*.16(2): 7-12.

Kastelic, J., Brito, L. (2012). Ultrasonography for monitoring reproductive function in the bull. *Reproduction in Domestic Animals*.47: 45-51.

Kessaris, D. N., Wasserman, P., Mellinger, B.C. (1995). Histopathological and cytopathological correlations of percutaneous testis biopsy and open testis biopsy in infertile men.*The Journal of Urology.* 153(4):1151-1155.

Leme, D., Papa, F. (2000). Cytological identification and quantification of testicular cell types using fine needle aspiration in horses. *Equine Veterinary Journal*. 32(5): 444-446.

Leme, D.P., Papa, F.O. (2010). How to perform and interpret testicular fine needle aspiration in stallions. *Journal of Equine Veterinary Science*.30(10): 590-596.

Lopate, C. (2012). The problem stud dog. *The Veterinary Clinics of North America. Small Animal Practice*. 42(3): 469-488.

Lopate, C., Threlfall, W., & Rosol, T. (1989). Histopathologic and gross effects of testicular biopsy in the dog. *Theriogenology, 32* (4), 585-602.

Mahajan, A.D., Ali, N.I., Walwalkar, S.J., Rege, J.D., Pathak, H.R. (1999). The role of fine-needle aspiration cytology of the testis in the diagnostic evaluation of infertility. *British Journal of Urology.* 84: 485-488.

Mallidis, C., Gordon Baker, H.W. (1994) Fine needle tissue aspiration biopsy of the testis. *Fertility and Sterility*. 61:367–75.

Masserdotti, C., Bonfanti, U., De Lorenzi, D., Tranquillo, M., Zanetti, O. (2005). Cytologic features of testicular tumours in dog. *Journal of Veterinary Medicine Series A*.52(7): 339-346.

McLachlan, R., Rajpert-De Meyts, E., Hoei-Hansen, C., De Kretser, D., Skakkebaek, N. (2007). Histological evaluation of the human testis: approaches to optimizing the clinical value of the assessment: mini review. *Human Reproduction.* 22(1): 2-16.

Mehrotra, R., Chaurasia, D. (2008). Fine needle aspiration cytology of the testis as the first-line diagnostic modality in azoospermia: a comparative study of cytology and histology. *Cytopathology*. 19(6): 363-368.

Meinhard E., McRae C.U., Chisholm G.D. (1973).Testicular biopsy in evaluation of male infertility.*British Medical Journal*.3: 577-581.

Memon, M. (2007). Common causes of male dog infertility. *Theriogenology*.68(3): 322-328.

Meng, M.V., Cha, I., Ljung, B.M., Turek, P.J. (2000). Relationship between classic histological pattern and sperm findings on fine needle aspiration map in infertile men. *Human Reproduction*. 15(9): 1973-1977.

Miyabayashi, T., Biller, D., Cooley, A. (1990). Ultrasonographic appearance of torsion of a testicular seminoma in a cryptorchid dog. *Journal of Small Animal Practice*. 31(8): 401-403.

Ober, C.P., Spaulding, K., Breitschwerdt, E.B., Malarkey, D.E., Hegarty, B.C. (2004). Orchitis in two dogs with Rocky Mountain spotted fever. *Veterinary Radiology and Ultrasound*.45(5): 458-465.

Odabas, Ã., Ugras, S., Aydin, S., Yilmaz, Y., Kemal Atilla, M. (1997). Assessment of testicular cytology by fine needle aspiration and the imprint technique: are they reliable diagnostic modalities? *British Journal of Urology*.79(3): 445-448.

Papic Z, Katona G, Skrabalo Z. (1988). The cytologic identification and quantification of testicular cell types: Reproducibility and relation to histologic findings in the diagnosis of male infertility. *Acta Cytologica*. 32: 697–706.

Pasqualotto FF, Sobreiro BP, Hallak J, Pasqualotto EB, Lucon AM. (2005). Sperm concentration and normal sperm morphology decrease and follicle-stimulating hormone level increases with age.*British Journal of Urology*.96(7): 1087-1091.

Peters, M., de Rooij, D., Teerds, K., van de Gaag, I., van Sluijs, F. (2001). Spermatogenesis and testicular tumours in ageing dogs. *Journal of Reproduction and Fertility Supplement*. 57: 419-421.

Quartuccio, M., Marino, G., Garufi, G., Cristarella, S., Zanghì, A. (2012). Sertoli cell tumors associated with feminizing syndrome and spermatic cord torsion in two cryptorchid dogs. *Journal of Veterinary Science*. 13(2): 207-209.

Qublan, H.S., Al-Jader, K.M., Al-kaisi, N.S., Alghoweri, A.S., Abu-Khait, S.A., Abu-Qamar, A.A., Haddadin, E. (2002). Fine needle aspiration cytology compared with open biopsy histology for the diagnosis of azoospermia. *Journal of Obstetrics and Gynaecology*. 22(5): 527-531.

Ragheb, D., Higgins, J. L. Jr. (2001). Ultrasonography of the scrotum: Technique, anatomy, and pathologic entities. *Journal of Ultrasound in Medicine.*21(2): 171-185.

Rammou–Kinia, R., Anagnostopoulou, I., Tassiopoulos, F., Lykourinas, M. (1999). Fine needle aspiration of the testis. Correlation between cytology and histology.*Acta Cytologica*. 43:991-998.

Rijsselaere, T., Maes, D., Hoflack, G., De Kruif, A., Van Soom, A. (2007). Effect of body weight, age and breeding history on canine sperm quality parameters measured by the Hamilton-Thorne analyser. *Reproduction in Domestic Animals.* 42(2): 143-148.

Romagnoli, S., Bonaccini, P., Stelletta, C., Garolla, A., Menegazzo, M., Foresta, C., Mollo, A., Milani, C., Gelli, D. (2009). Clinical use of testicular fine needle aspiration cytology in oligozoospermic and azoospermic dogs. *Reproduction in Domestic Animals*.44(s2): 329-333.

Root Kustritz, M.V. (2006). Collection of tissue and culture samples from the canine reproductive tract. *Theriogenology*. 66(3): 567-574.

Root Kustritz, M. V. (2007). The value of canine semen evaluation for practitioners. *Theriogenology*. 68(3): 329-337.

Rosenlund, B., Kvist, U., Plöen, L., Rozell, B. L., Sjöblom, P., Hillensjö, T. (1998). A comparison between open and percutaneous needle biopsies in men with azoospermia.*Human Reproduction.*13 (5): 1266-1271.

Russell, L.D., Ren, H.P., Hikim, I.S., Schulze, W., Hikim, A.P. (1990). A comparative study in twelve mammalian species of volume densities, volumes, and numerical densities of selected testis components, emphasizing those related to the Sertoli cell. *The American Journal of Anatomy.*188(1): 21-30.

Santos, M., Marcos, R., Caniatti, M. (2010). Cytologic study of normal canine testis. *Theriogenology*. 73(2): 208-214.

Saxon, P., Badler, R.L., Desser, T.S., Tublin, M.E., Katz, D.S. (2012).Segmental testicular infarction: report of seven new cases and literature review. *Emergency Radiology*. 19(3): 217-223.

Schenck, U., Schill, W.B. (1988). Cytology of the human seminiferous epithelium. *Acta Cytologica.* 32: 689-396.

Schubert, C.L., Seager, S.W.J. (1991). Semen collection and evaluation for the assessment of fertility parameter in the male Dalmatian. *Canine Practice*. 16(5): 17-21.

Schurich, M., Aigner, F., Frauscher, F., Pallwein, L. (2009). The role of ultrasound in assessment of male fertility. *European Journal of Obstetrics, Gynecology, and Reproductive Biology, 144*(1): 192-198.

Seager, S. W. J., Schubert, C.L. (1996). Semen collection and evaluation for the clinical assessment of fertility parameters in the male Rottweiler. *Canine Practice*. 21(6): 30-34.

Soares, J.M., Avelar, G.F., França, L.R. (2009). The seminiferous epithelium cycle and its duration in different breeds of dog (Canis familiaris). *Journal of Anatomy*. 215(4): 462-471.

Souza, F.F., Leme, D.P., Uechi, E., Trinca, L.A., Lopes, M.D. (2004). Evaluation of testicular fine needle aspiration cytology and serum testosterone levels in dogs. *Brazilian Journal of Veterinary Research and Animal Science.*41(2): 98-105.

Srivastava, A., Raghavendran, M., Jain, M., Gupta, S., Chaudhary, H. (2004). Fine-needle aspiration cytology of the testis: Can it be a single diagnostic modality in azoospermia? *Urologia Internationale*. 73(1): 23-27.

Stelletta, C., Juyena, N., Ponce Salazar, D., Ruiz, J., Gutierrez, G. (2011). Testicular cytology of alpaca: Comparison between impressed and smeared slides. *Animal Reproduction Science*.125(1): 133-137.

Turek, P.J., Cha, I., Ljung, B.M. (1997). Systematic fine-needle aspiration of the testis: correlation to biopsy and results of organ mapping for mature sperm in azoospermic men. *Urology*.49(5): 743-748.

Wiess, D.B., Gottschalk-Sabag, S., Folb-Zacharow, N. (1996).Quantitative analysis of spermatogenesis in fine-needle aspirates of the testis.*Harefuah*. 130(6): 370-375.

Yildiz-Aktas, I.Z., Monaco, S.E., Khalbuss, W.E., Parwani, A.V., Jaffe, T.M., Pantanowitz, L. (2011).Testicular touch preparation cytology in the evaluation of male infertility.*Cytojournal*. 8: 24.