

Evaluation testicular fine needle aspiration cytology and serum testosterone levels in dogs

Avaliação da função testicular utilizando a citologia aspirativa e testosterona sérica em cães

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Abstract

In order to verify alternative clinical approach in the reproductive evaluation of 4 adult dogs, the fine needle aspiration cytology (FNAC) and serum testosterone levels were used together with testicular volume measurement and semen analysis. FNAC was performed in both testes and serum testosterone concentrations were assayed in regular intervals during a 24h period. Results of semen analysis and FNAC were normal in dogs 1 and 3. In dog 2, small testes, poor semen quality, a high percentage of Sertoli cells and early spermatids were found suggesting testicular degeneration. In dog 4, a small right testicle, poor semen quality with low sperm concentration and an uncounted amount of spermatozoa in the FNAC indicated testicular degeneration due to an obstructive lesion; whereas high percentage of distal droplets, thicker left epididymis and normal FNAC of the left testis suggested a slow sperm transit. Testosterone circadian rhythm was clear in 3 of 4 dogs, although concentrations were low. Testicular volume, semen analysis and testicular FNAC could provide valuable information about spermatogenesis. In contrast, serum testosterone concentration was not clearly correlated with any reproductive characteristic of those dogs.

Key-words:

Dog.
Semen.
Testicular fine needle aspiration.
Serum testosterone.

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Recebido para publicação: 26/08/2003
Aprovado para publicação: 25/03/2004

Introduction

Canine fertilization failure can be a result of numerous factors including those which are either female or male in origin. There has been an advance in assessing female infertility, however evaluation of male infertility is still limited.¹

Reproductive analysis of the canine male consists of a physical examination, semen evaluation, measurement of endocrine parameters, testicular biopsy, epididymal markers and epididymal aspiration.²

Semen evaluation has always been

indicated in cases of infertile or subfertile dogs.³ More recently, with the development of noninvasive techniques such computer-assisted semen analysis (CASA) have been emphasized for evaluating potential fertility of males.^{4,5}

However, testicular biopsy can provide information on testicular function that cannot be obtained by any other method⁶. Studies of testicular biopsy in dogs have shown that its use is a safe diagnostic procedure⁷ and it does not cause interference with spermatogenesis⁸. Different testicular biopsy techniques have been recommended to provide information regarding the integrity of seminiferous epithelium in

infertile or subfertile male dogs.^{8,9,10,11}

Among testicular biopsy techniques, fine needle aspiration cytology (FNAC) has proven to be a less painful, simple and minimally invasive procedure. It allows evaluation of cytological parameters of seminiferous epithelium¹⁶ and its clinical use in dogs has been reported to evaluate seminiferous epithelium of dogs.^{1,13} There were no negative effects on sperm quality after testicular biopsies.^{7,8}

Although hormone determinations may be included in the male breeding soundness exam, the relationship between endocrine abnormalities and reproductive malfunctions has not been fully studied.¹⁴ Moreover, correlation between testosterone concentrations and semen parameters has not been determined in dogs.¹

The objective of this study was to evaluate the use of testicular FNAC in association with serum testosterone concentrations, testicular size and semen analysis in clinical cases of four sexually mature male dogs.

Material and Methods

Data from 4 adult cross bred dogs of unknown breeding history were collected at the Department of Animal Reproduction and Veterinary Radiology, FMVZ, UNESP, Campus of Botucatu, São Paulo, Brazil. The dogs ranged from 12 to 25 kilograms in body weight. Brucellosis (Canine Brucellosis Antibody Test Kit, D-Tec® CB, Synbiotics Corporation, USA) and leptospirosis (Microscopic Serum-Agglutination) serum tests for these dogs were negative.

Semen analysis

After a training period of 2 weeks, one ejaculated were collected from each dog by digital manipulation of the penis. Characteristics of each ejaculated such volume, spermatic concentration, spermatic vigor, progressive motility and spermatic

morphology were assessed¹⁵. Volume (mL) was measured in a graduated cylinder. Progressive motility (%) and spermatic vigor (0 to 5) were estimated in a drop of semen with a cover slip on a warmed (37°C) slide at 400x magnifications in light microscopy. Spermatic concentration ($\times 10^6$ /mL) was determined by diluting the semen 1:20 and placing the diluted sample in a hemocytometer chamber to count the sperm cells. The spermatic morphology (%) of 200 cells was evaluated on Karras stained smears¹⁶.

Blood samples and radioimmunoassay

Jugular blood samples were collected into vacutainer tubes with no anticoagulants at 4h intervals during a 24h period. Samples were transported immediately to the laboratory and serum was separated by centrifugation (800 g for 15 minutes) and frozen at -20°C . Testosterone concentration was assayed by using a commercially available RIA kit (COAT-A-COUNT®, Total Testosterone, DPC, Los Angeles, CA), which was validated for dog serum.¹⁷ The assay sensitivity was 0.14 nmol/L and the intra-assay CV was 5.8%.

Size and fine needle aspiration cytology of the tests

For testicular evaluation and the FNAC procedure, the dogs were positioned in dorsal recumbency. Testicles and epididymides were palpated for consistency and abnormalities were recorded. A caliper was used for measuring both testes on each dog. Height, width and length were recorded and the following formula for elliptical structures was applied to obtain testicular volumes (TV): $TV = 4/3\pi \times \text{height}/2 \times \text{width}/2 \times \text{length}/2$.¹⁸ After routine surgical preparation of the scrotal skin, a 21-gauge needle connected to a 10 mL-syringe was halfway inserted into each testis, which were gently aspirated avoiding the epididymidis. The withdrawal fluid was smeared, air-dried, stained with Giemsa and examined under light microscopy 1,250x magnifications. Two hundred consecutive

spermatogenic cells and the Sertoli cells between germ cells were counted on each smear. The following structures were identified using the criteria described by Schenk and Schill¹⁹ and Leme and Papa¹³: spermatogonia, primary spermatocytes, secondary spermatocytes, early and late spermatids, spermatozoa and Sertoli cells. Dogs were carefully observed for any clinical sign of pain, swelling, hematoma formation or fever after testicular aspiration.

Statistical analysis

Data from semen analysis, testicular volume and FNAC quantification were analyzed and described qualitatively for each dog. For each dog, overall mean, standard deviation (SD) and coefficient of variation (CV) values of testosterone concentration were calculated²⁰. Testosterone concentrations that were below the sensitivity of the assay were not considered in the analysis. The regression analysis was used to correlate the serum testosterone concentrations and data of seminal analysis calculated in Excel software (Microsoft ® 2000).

Results and Discussion

Values including the testicular volume, body weight and semen analysis are presented in table 1 and 2, respectively. Testosterone concentration at the different time points from each dog, as well as individual mean, SD and CV values are showed in table 3. Identified cell types (Figures 1, 2, 3 and 4) and quantification of those cells by FNAC are also presented in table 4.

The dogs were calm and no signs of pain were visualized during fine needle aspiration of the testis. Therefore, no sedatives were necessary. No clinical changes were observed in these dogs during the procedure and there were complications after FNAC.

With the use of FNAC, it was possible to obtain enough material from both testis to identify and quantify the spermatogenic cells and Sertoli cells in 3 dogs (Dog 1, 3 and 4). In

Table 1

Testicular volume (cm³) and body weight (kg) from 4 mature dogs (R: Right Testis and L: Left Testis). Botucatu, SP, 2001

Dog	Body Weight (kg)	Testicular Volume (cm ³)	
		R	L
1	25	11.76	11.68
2	12	2.04	3.52
3	12	7.94	8.46
4	15	2.8	8.7

Table 3

Testosterone concentration (nmol/L) at different time points during a 24h period from 4 sexually mature male dogs. Individual mean, SD and CV (%) values. Botucatu, SP, 2001

Time Points	Dog			
	1	2	3	4
8:00 am	12.33	0.98	0 ^a	7.41
12:00 am	0 ^a	0.04 ^a	0 ^a	4.81
4:00 pm	1.08	1.44	0.31	4.23
8:00 pm	4.08	2.19	1.15	4.24
12:00 pm	1.98	0.23	0.86	6.53
4:00 am	0 ^a	0.31	0 ^a	6.31
8:00 am	2.41	0.36	1.91	4.75
Mean	4.38	0.92	1.06	5.47
SD	3.73	0.78	0.66	1.26
CV (%)	85	85	63	23

^aValues below the sensitivity of the assay were not considered in the analysis

dog 2, the left testicle smear had high blood contamination making it impossible to quantify those cells.

In dog 1, clinical examination of the testis and epididymides showed that the scrotal skin was thicker than normal. Testicular volume was compatible with its body weight.²¹ The thickness of scrotal skin apparently did not affect the testicular volume. Semen quality was normal according to Johnston, Root Kustritz and Olson.¹⁰ Although the number of a given cell type in a smear do not have much significance. The relationship among the testicular cell types and their deviation from normal values are of special importance for the FNAC diagnoses.²² In this dog, percentages from spermatogonia to late spermatids were increased showing a normal evolution of the spermatogenic

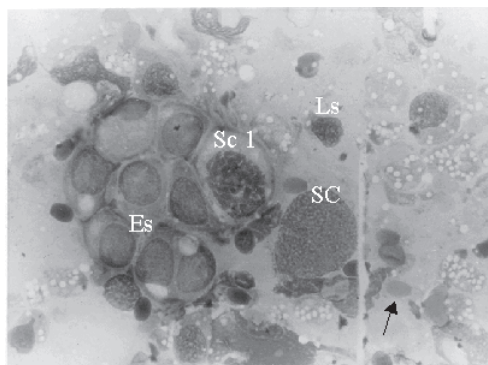


Figure 1
Testicular fine needle aspiration from a mature dog (dog 1; right testicle). Sc 1 = Primary spermatocyte; SC = Sertoli Cell nucleus; Es = Early spermatid; Ls = Late spermatid; black arrow = spermatozoon. Giemsa 1,250x

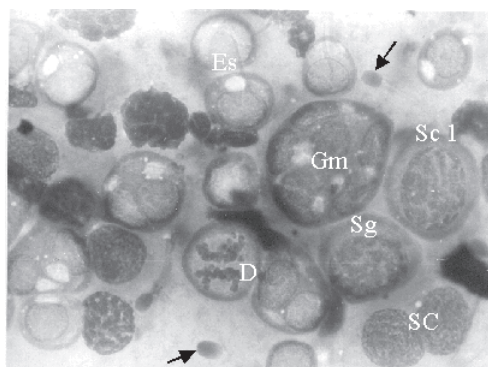


Figure 2
Testicular fine needle aspiration from a mature dog (dog 2; right testicle). Sc 1 = Primary spermatocyte; Es = Early spermatids; SC = Sertoli Cell nuclei; Gm = Giant multinucleated cell; Sg = Spermatogonium; D = Cellular division; black arrow = spermatozoon. Giemsa 1,250x

process. The low percentage of secondary spermatids observed in FNAC exams is due to the short life span of this cell type.²² The relationship between germ cell and Sertoli cell numbers has been studied as an indicator of the spermatogenesis efficiency in different species.^{22,23,24,25,26} Low percentages of Sertoli cells indicate that the cell population of seminiferous epithelium is composed primarily by germ cells. This was observed in this case (Table 4). Testosterone concentrations throughout a 24h period were normal according to the values described by Olson²⁷ (1.38 to 34.7 nmol/L), however there was an important variation among time points (Table 3).

In Dog 2, small testicular volume²¹ in association with poor semen quality were compatible with low sperm production.^{10,28} The high amount of blood obtained from the left testicle in the FNAC was in agreement with Rajwanshi et al.²⁹, which described that inadequate smears could be contaminated with blood. Besides that, the left testicle smear showed normal cellular components of spermatogenesis. However, multinucleated giant cells at the primary spermatocyte stage were abnormally present. This could be due to an increase in testicular temperature that can rapidly induce failure of the primary spermatocyte to differentiate leading to multinucleated cell formations. Generally, the presence of these cells evidenced testicular degeneration.³⁰ FNAC of the right testicle showed a high percentage of Sertoli cells

Table 2

Volume of second fraction (mL), sperm progressive motility (%), spermatic vigor (0 to 5), sperm concentration (x 10⁶/mL), normal sperm (%) and major and minor defects (%) from 4 sexually mature male dogs. Botucatu, SP, 2001

Dog	Volume (mL)	Progressive Motility (%)	Vigor (0 to 5)	Concentration (x 10 ⁶ /mL)	Normal Sperm (%)	Major Defects (%)	Minor Defects (%)
1	2	90	3 to 4	368	90.5	3.5	6
2	1	65 to 70	2 to 3	12.6	42.5	40	17.5
3	2.5	90 to 95	4 to 5	446	57	20.5	22.5
4	0.5	10	0 to 1	0.95	42	12	46

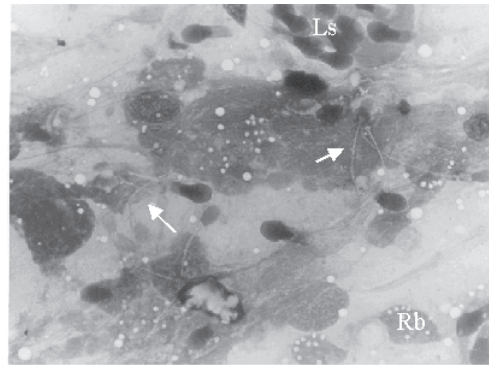


Figure 3
Testicular fine needle aspiration from a mature dog (dog 4; right testicle). Ls = Late spermatids; Rb = Residual body; white arrow = accumulated spermatozoa. Giemsa 1,250x

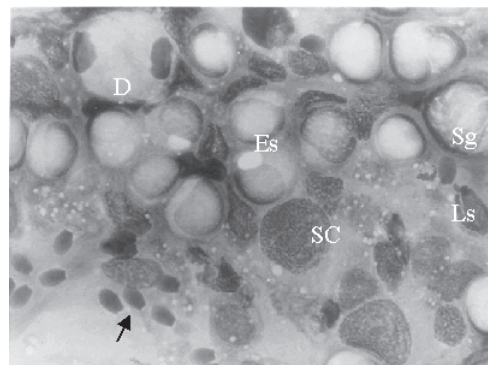


Figure 4
Testicular fine needle aspiration from a mature dog (dog 4; left testicle). Sg = Spermatogonium ; Es = Early spermatids; Ls = Late spermatid; SC = Sertoli Cell nucleus; D = Cellular division; black arrow = spermatozoa. Giemsa 1,250x

(65%) and a great deviation for early spermatids (78%). Multinucleated cells of spermatid stage were also observed in this smear. The increased number of Sertoli cells is often associated with a quantitative reduction of the germ cell line; furthermore the high percentage of late spermatids suggests the existence of a partial maturation arrest at spermatid level. In this case, the presence of both multinucleated cells and the increased number of morphologically abnormal sperm were important to reveal inadequate spermiogenesis¹². However, 3 months later (unpublished data) this dog showed a great improvement of sperm concentration (12.6×10^6 cells/mL versus 148×10^6 cells/mL). These results indicate recovery of testicular degeneration. Testosterone serum levels of this dog were much lower than normal²⁷ in almost all observed time points, but there seemed to be circadian rhythm.

In Dog 3, testicular volume was consistent with its body weight²¹. Semen evaluation was also normal¹⁵, although there were a high number of morphologically abnormal spermatozoa. Most abnormalities were proximal droplets. In this case, spermiogenesis could be affected in some time periods, but not enough to decrease total sperm production. This was also observed by FNAC results that showed normal percentage of germ cells and Sertoli cells.¹² Testosterone serum concentrations of this dog were low in all observed time points.²⁷

Table 4

Quantification of testicular cell types by FNAC in 4 mature dogs (R: Right Testis and L: Left Testis). Botucatu, SP, 2001

Cell Types	Dog							
	1		2		3		4	
	R (%)	L (%)	R (%)	L (%) ^a	R (%)	L (%)	R (%)	L (%)
Spermatogonia	5.5	1	4.5	—	3	4	2	4
Primary Spermatocytes	9	7.5	3.5	—	2.5	1.5	3.5	6.5
Secondary Spermatocytes	0.5	0	0.5	—	0.5	0.5	1.5	0
Early Spermatids	28	43	78.8	—	37	41	26	44
Late Spermatids	49.5	30.5	6	—	14	20	60	24.5
Spermatozoa	6.5	18	6	—	43	33	7	21
Sertoli Cells	4	5	65	—	1	16	7	11
Giant Multinucleated Cells	Absent		Present		Absent		Absent	

^aBlood contamination

In Dog 4, the left testicular volume was consistent with its body weight, whereas the right testicle was much smaller than normal.²¹ The right head and tail of the epididymis were abnormally thick. Poor quality semen was clearly evident by a low sperm concentration and a high percentage of morphologically abnormal spermatozoa. In FNAC, different features were found for each testis. In the right testicle, a much higher percentage of late spermatids and a lower percentage of spermatozoa indicated abnormal spermiogenesis. However, it was possible to identify a large and uncountable amount of spermatozoa on the right testicle smear (Figure 2). This suggests an occlusive lesion with testicular degeneration.^{1,31} In the left testicle, FNAC showed normal percentage of germ cells and Sertoli cells. However, the low sperm concentration and the high percentage of distal droplets were compatible with a delay in sperm transit. The thicker epididymal head and tail could be consistent with this. Testosterone concentrations were normal.²⁷

In this study, serum testosterone concentration was not correlated with semen analysis by regression analysis. However, sexual characteristics such as spermatogenesis, libido and ejaculation that require normal testosterone levels were present in these dogs. It is well known that peripheral venous level

of testosterone is not a reliable guide for the intratesticular concentration of testosterone.¹⁴ The results confirm that serum testosterone concentration is not a reliable method for evaluating spermatogenesis in the dog. Furthermore, there are great individual variations in testosterone levels between the same dog at different times of the day, which showed the pulsatile pattern of testosterone secretion. This is demonstrated by the high individual CV values. According to the results presented by Fukuda³², testosterone pulses were representative of a circadian rhythm observed in dogs 1, 2 and 3. Additional investigation of testicular steroids quantification in peripheral blood compared with semen analysis should be done in dogs to gain information regarding endocrine testicular function.

In conclusion, this study has shown that clinical exam, testicular volume, semen analysis and testicular FNAC were valuable and very helpful tools in canine male reproductive evaluation. In addition, these results demonstrated the safety of testicular FNAC.

Acknowledgments

Financial support from FAPESP: 98/16388-6, 98/14740-4.

Resumo

Com o objetivo de avaliar a função reprodutiva de 4 cães machos, adultos, a citologia aspirativa com agulha fina (FNAC) e as concentrações de testosterona sérica foram usadas em associação a determinação do volume testicular e análise do sêmen. FNAC foi realizada em ambos os testículos e as concentrações de testosterona foram determinadas durante 24 horas, em intervalos regulares. Os resultados da análise do sêmen se encontraram dentro do padrão para a espécie nos cães 1 e 3. O cão 2 apresentou testículos pequenos, baixa qualidade seminal, alta porcentagem de células de Sertoli e espermátides iniciais sugerindo uma degeneração testicular. No cão 4 observou-se uma degeneração testicular do lado direito consequência de um processo obstrutivo mostrada pela diminuição do testículo, baixa qualidade do sêmen evidenciada pela baixa concentração espermática e incontável número de espermatozoides na FNAC; uma diminuição do trânsito espermático foi observada no testículo esquerdo, com espessamento do epidídimo, alta porcentagem de gotas distais na análise seminal, porém resultados normais na FNAC.

Palavras-chave:

Cão.
Sêmen.
Punção aspirativa do testículo com agulha fina.
Testosterona Sérica.

O ritmo circadiano da testosterona foi claro nos cães 3 e 4, entretanto as concentrações encontraram-se próximas ao limite inferior. O volume testicular, a análise do sêmen e a FNAC testicular podem fornecer informações valiosas sobre a espermatogênese. Entretanto, as concentrações séricas de testosterona não são claramente correlacionadas as características reprodutivas nesses cães.

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