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EVALUATION OF FINE NEEDLE ASPIRATION CYTOLOGY (FNAC) AS AN AID FOR DIAGNOSIS OF TESTICULAR AFFECTIONS IN STALLIONS

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SUMMARY

This study included thirty stallions (4 to 12 years old) admitted to the Surgery clinic, Faculty of Veterinary Medicine, Cairo University for castration. A preliminary general examination in conjunction with visual inspection and mannual palpation of both testicles were carried out for each animal. Fine needle aspiration cytology (FNAC) of the testis was performed in these animals and the cytological evaluation was recorded. After castration, testicular samples were collected from the same testis for histopathological investigation. Results of histopathological investigation were compared with those of fine needle aspiration cytology. The present study indicated that, FNAC is a non-invasive practical technique that can be used satisfactorily for diagnosis of testicular affections and infertility in stallions.

INTRODUCTION

Classification of male infertility was based on pretesticular, testicular, and post-testicular causes. Pretesticular causes included hypogonadotropism, excessive estrogen, excessive androgen, glucocorticoid excess and hypothyroidism. Testicular affections comprised maturation arrest, hypospermatogenesis, chromosomal abnormalities, cryptorchidism, hypoplasia, degeneration and orchitis. Post-testicular causes involved blockage of ducts leaving the testis, defective spermatozoal maturation or storage in the epididymis, and biochemically abnormal seminal plasma (Wong et al., 1973; Parkinson, 1996; Watson, 1998).

Infertility in stallions can be assessed by one or more of the following investigations; clinical examination, seminal evaluation, spermatozoal antibody determination, endocrinologic

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investiagtions, karyotyping, ultrasonography and testicular biopsy (Gray, 1996; Watson, 1998).

Testicular biopsy is one of the diagnostic tools of male infertility. It is helpful in the evaluation of germinal cell aplasia, germinal cell arrest, hypogonadotropic eunuchoidism, hypospermatogenesis, Klinefelter's syndrome, and hypogonadotropism (Larsen, 1977; Cohen, 1984; Parkinson, 1996; Watson, 1998). However, its invasiveness, traumatizing effects, bleeding from the scrotum and pain reaction have limited its use (Meingard et al., 1977; Siiberg and Rodriguez-Rigau, 1981; Parkinson, 1996).

Other techniques of testicular aspiration biopsies were proposed to be less invasive procedures but their high traumatizating effects limited their application (Nseyo et al., 1984).

Fine needle aspiration cytology (FNAC) has been proposed as a non-invasive technique for cytological evaluation of testicular affections (Person et al., 1971; Gottschalk-Sabag et al., 1993; Mallidis and Baker, 1994). Foresta and collegues, (1992); Khattab et al., (1993) and Craft et al., (1997) reported that testicular FNAC is a highly reproducible diagnostic procedure of human infertility. However, there is no much experience concerning the use of this technique in veterinary practice (Finco, 1977; Nseyo et al., 1984; Sundqvist and Parvinen, 1986).

The aim of the present work is to evaluate whether testicular FNAC can provide satisfactory diagnostic cytological material compared with the routine histopathological investigations in normal and affected testes.

MATERIAL AND METHODS

Thirty native stallions (4-12 years old) were included in this study. All the animals were introduced to the surgery clinic, Faculty of Veterinary Medicine, Cairo University for castration. Diagnosis of testicular affections was based on history and clinical findings. History included sexual activity and previous genital tract affections. The heart rate, respiratory rate, and rectal temperature were also recorded. The testis were examined for size (scrotal circumference, testis length, breadth and thickness), symmetry, consistency, position and movability in the scrotum.

Testicular aspirates were obtained by Fine Needle Aspiration (FNA) under the effect of light chloral hydrate narcosis (10%). The scrotum was thoroughly washed with povidone-iodine, scrubbed and rinsed. Using 22 G. long needle, the secured testis (right and left) was percutaneously punctured. The piston of the syring was retracted, creating a vacuum, and the needle was moved back and forth and rotated 360 around its longitudinal axis for 5 times. Aspiration was done following each rotation. The site of puncture corresponded to the middle portion of the testis on the opposite side of the epididymis. After removal of the needle, direct finger pressure was applied. The aspirated material was used to prepare 2 smears which were allowed to air dry, fixed with methyl alcohol and then stained by May-Grunwald Giemsa (MGG) stain (Coles, 1986).

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Smears were screened for spermatogenic and Sertoli cells. The cytological features of 200 cells were qualitatively evaluated on the basis of their morphological and staining aspects. Spermatogonia (dark and pale), primary spermatocytes, secondary spermatocytes, early spermatids, late spermatids, and spermatozoa were identified, quantitatively expressed as percentages of the total. The percentages of spermatozoa (spermatic index) indicated the number of spermatozoa per 100 spermatogenic cells. Sertoli index represented the number of Sertoli cells per 100 spermatogenic cells (Raiwanshi et al., 1991; Foresta et al., 1992).

After one week, castration was performed under chloral hydrate general narcosis. Prophylactic dose of antitetanic serum, systemic course of antibiotics, forced exercise and hydrotherapy of the inguinal region were contuined for three days post-operative. The removed testicles were examined grossly for possible complications of FNAC. Testicular samples were processed by the routine paraffin embedding technique and stained by Haematoxylin and Eosin (Bancroft and Cook, 1984) for histopathological investigation.

RESULTS

All the thirty stallions included in the present work were vicious with aggressive sexual behaviour. They were alert and active with apparently normal general health condition (rectal temperature ranged between 37.5°C-38.2°C, heart rate 43-65/min and respiratory rate 12-16/min). Examination of the scrotum revealed pathological conditions in four animals (13.3%). The testicles of one stallion were not easily palpable, non movable with accumulation of fluids in the scrotum. Exploratory puncture revealed sangiunous fluid. A preliminary diagnosis of a hydrocele was recorded. The other three stallions showed small testicles and preliminary diagnosed as unilateral atrophy in one and bilateral degeneration in two stallions (Table 1).

Table 1: Testicular measurements of the normal and affected stallions:

No. of animals	Scrotal circumf. (cm)	Right testis measurement (cm)			Left testis measurement (cm)			Testicular palpation	Preliminary diagnosis
		L	B	T	L	B	T		
26	34.22±3.54	8.53±1.34	5.52±0.82	6.32±0.53	9.13±0.78	5.64±0.54	6.21±0.53	Normal texture	Apparently normal animals
affected cases									
one	39.80	14.00	7.50	8.00	18.00	9.50	11.50	Not easily palpable	Hydrocele
one	26.40	5.50	3.70	4.30	8.50	5.00	6.50	Firm	Unilateral atrophy
two	29.75±1.76	7.30±0.70	4.25±0.35	4.95±0.49	6.30±2.12	4.20±0.28	5.0±0.28	Flabby (soft)	Bilateral degeneration

±SD L=length B=breadth T=thickness

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Table 2: Mean percentages of spermatogenic cells and indices observed in smears of normal and affected testicles (±SE).

Type of cells	Group 1 (n=26)						Group 2 (n=4) Grossly affected		
	Group 1a (n=23) Microscopically normal			Group 1b (n=3) Microscopically normal					
	R.T	L.T	Mean	R.T	L.T	Mean	R.T	L.T	Mean
Pale spermatogonia	5.50±0.55	3.30±0.04	4.40±0.45a	3.33±0.32	4.67±0.86	3.83±0.68	1.14±0.45	1.29±0.55	1.21±0.49b
Dark spermatogonia	3.40±0.36	4.80±0.80	4.1±0.58a	4.33±1.45	4.67±0.88	4.50±1.10	0.71±0.44	0.86±0.34	0.79±0.29b
Primary spermatogonia	5.40±0.55	6.00±0.50	5.70±0.52a	3.33±0.88	3.00±1.73	3.17±1.20c	0.86±0.45	1.71±0.55	1.29±0.53b
Secondary spermatogonia	2.90±0.28	2.60±0.36	2.75±0.32a	0.67±0.33	1.00±0.58	0.83±0.43b	0.00±0.00	0.00±0.00	0.00±0.00b
Early spermatids	22.30±1.02	22.30±1.34	22..30±1.18a	15.33±2/40	11.33±0.66	13.33±2.02b	11.0±1.22	9.70±1.67	10.36±1.45b
Late spermatids	20.30±1.20	17.90±0.59	19.10±0.89	13.00±2.00	16.00±1.53	14.50±1.85b	9.43±0.99	8.14±1.13	8.79±1.10b
Spermatic index	25.50±1.60	28.00±0.80	26.70±1.24a	18.33±2.84	22.67±0.88	18.50±2.33b	11.43±1.38	10.14±1.32	10.79±1.33b
Sertoli index	18.40±0.90	16.60±0.95	17.50±0.93a	41.67±2.73	37.00±1.53	40.33±2.47b	65.43±2.32	68.16±2.13	66.79±2.25b

At the same raw: a-b= P<0.001 & a-c= P<0.05

Qualitative cytological examination of the smears obtained from stallions revealed that nuclei of spermatogonia had diameters of 18 to 22µM and finely threaded chromatin. *Pale and dark spermatogonia* were distinguished according to their chromatin density (Fig. 1, a and b). Pale- stained chromatin, with a deeper-stained semilunar peripheral area and a washed-out central area were cellular characterisitics of *Pale spermatogonia. Dark spermatogonia* exhibited deeply stained, dark-violet chromatin with a denser and deep-stained semilunar area, located peripheral to the nuclear membrane. Cytoplasm of both types of cells was scanty to moderate and less basophilic than that of mature cells. Spermatogonia had single, round or oval nuclei. Moreover, multinucleated cells (undergoing mitosis) were common in the field.

Primary spermatocytes were clearly identified by their nuclear structure. They had a round shape, 19 to 24 µM in diameter, deeply stained threaded chromatin and an eccentric nucleolus (Fig. 2). Binucleated primary spermatocytes (undergoing meiosis) connected by a cytoplasmic bridge, were common (Fig. 3). The cytoplasm appeared scanty but more basophilic, especially in the periphery of the cell.

The nuclear mass of the secondary spermatocytes was approximately half that of primary spermatocytes (10 to 12µM). Their chromatin pattern appeared uniformly and finely granular (Fig. 3). The nucleus was round, centrally placed and surrounded by a slightly basophilic cytoplasm. Each cell was binucleated to give rise two spermatids (Fig. 4).

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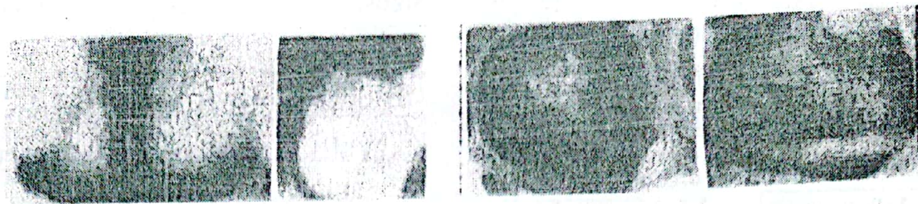

Fig. 1: a) Pale spermatogonia b) Dark spermatogonia

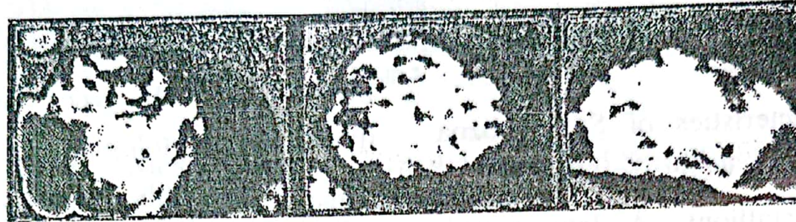

Fig. 2: Primary spermatocyte "differentstages of meiosis"

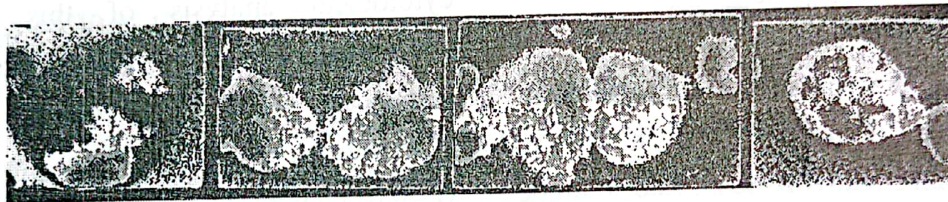

Fig. 3: Progression of meiotic division of the primary speramtocyte till the formation of secondary spermatocyte.

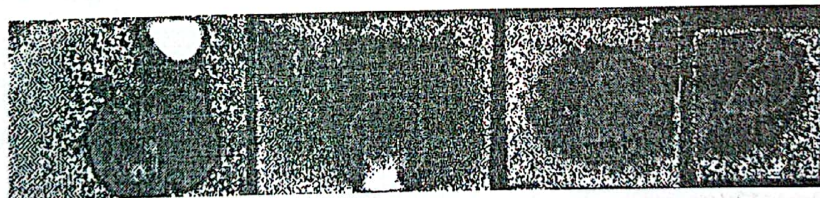

Fig. 4: Different developmental stages of early spermatids.

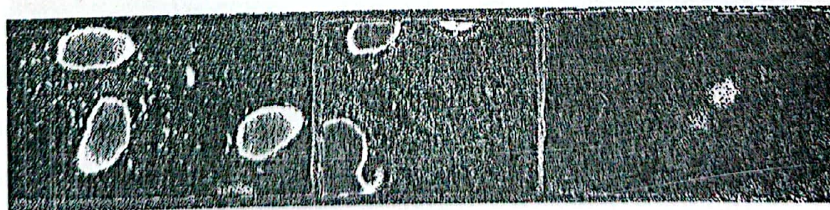

Fig. 5 : Different developmental stages of late spermatids and spermatozoa.

CYTOLOGIC FEATURES OF DIFFERENT SPERMATOGENIC CELLS.

Early spermatids were twice as small as secondary spermatocytes. They had a round or slightly triangular nucleus, eccentrically located and showed a uniformly, finely granular chromatin without nucleoli (Fig. 4). *Late spermatids* showed a smaller, elongated shape and uniformly deeply darker chromatin. The cytoplasm of these cells appeared abundant (sometimes vacuolized) and gathered on the same side of the growing tail (Fig. 5).

Morphological characteristics of Spermatozoa (head, neck, and tail) were well-defined in smears of normal stallions. A few smears showed spermatozoa with small cytoplasmic residue on the neck region (Fig. 5).

Sertoli cells showed a round or a kidney-shaped nucleus (12-15μm diameter), with a regular finely granular chromatin. It possessed a single conspicuous, eccentrically located nucleolus. The cytoplasm appeared large, pale or slightly basophilic with a triangular shape, illdefined borders and containing tiny vacuoles of various sizes.

Histopathological investigations revealed that the apprently normal animals (26/30=86.7%, group 1) divided into; animals with normal microscopical findings (23/26=88.8%, group 1 a) and animals with abnormal microscopical findings (3/26=11.5, group 1b). Group 1a showed intact seminiferous tubules with the presence of all types of germ cells. The quantitative cytological analysis of this group was listed in table 2. Group 1b showed different forms of degenerative changes in the seminiferous tubules (Fig. 6). The quantitative cytological analysis of this group, in a comparison with normal animals, revealed significant ($P<0.05$) reduction in primary spermatocytes. Also, there was significant ($P<0.01$) decrease in secondary spermatocytes, early and late spermatids and spermatic index. While the Sertoli index showed significant ($P<0.01$) increase.

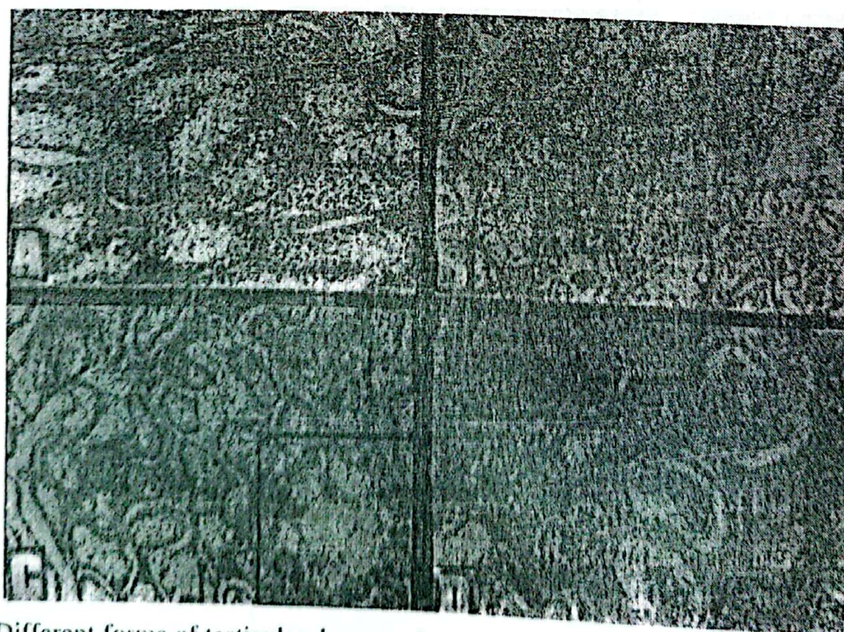

Fig (6): Different forms of testicular degeneration in the affected animals; mild degeneration (a), germ cell desquamation (b), vacuolation of tubular epithelium (c), fiborosis and calcification (d).

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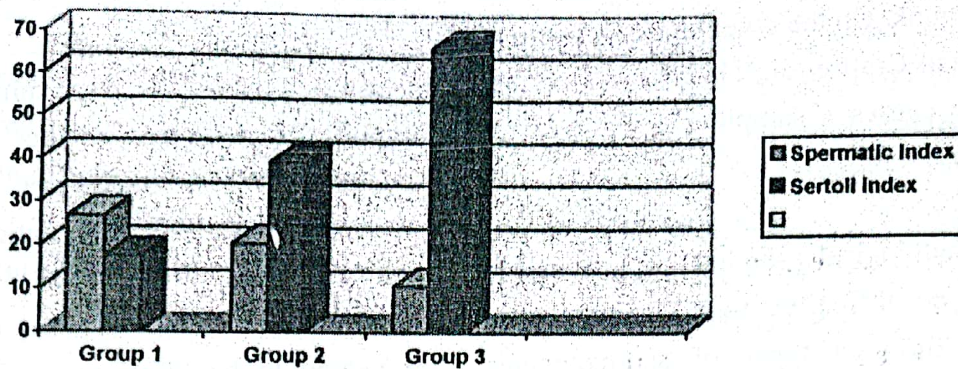

Fig. (7): Mean values of spermatic index and Sertoli index in the three groups of stallions.

Regarding the histopathological results of the affected stallions (4/30 = 13.3%, group2), there was a degenerative changes in the seminiferous tubules, thickness of basement membranes, vacuolation of tubular epithelium, decreased number of all types of germ cells, germ cell desquamation, fibrosis and calcification (Fig. 6).

comparing with the normal stallions, the quantitative cytological analysis revealed significant ($P < 0.01$) reduction in the mean percentage of all stages of spermatogenic cells and spermatic index with significant ($P < 0.01$) increased in Sertoli index (Table 2 and Fig 7).

After castration, no haematomas were observed in the sites of needle aspirations. All animals were discharged three days after operations without complications.

DISCUSSION

The first step in the present work was to devise a practical diagnostic technique of testicular affections in stallions. Clinical examination and preliminary diagnosis revealed that 13.3% of stallions showed gross testicular affections. By application of fine needle aspiration cytology, this percentage has been changed to 23.3%. Moreover, this incidence might not reflect the actual breeding potential and fertility in native equine population.

In the present study, the testicular dimensions and scrotal circumference were more or less simillar to that obtained by Hemeida et al., (1980). As reported by Hemeida et al., (1980) these parameters were not a fair indication of testicular spermatid production. FNAC was used to obtain a material from apparently normal stallions and few affected clinical cases. The use of a small needle of 20-22 gauge to collect the cells did not cause any measurable damage to the testis. This was confirmed after castration where there was no haematomas or testicular damage in the sites of needle aspirations. These results were in agreement with Sundqvist and Parvinen, (1986) who reported that FNAC had no harmful

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effects on libido or general behaviour and they indicated it as the method of choice in breeding selection in mink. On the other hand, DeVento et al., (1992) and Craft et al., (1977) reported that open biopsy causes a sampling damage but it was transitory.

Qualitative cytological examination appeared to be easy and no difficulties were encountered in recognizing different types of spermatogenic cells. These results were coincide with those of Foresta et al., (1992). However, Schenck and Schill, (1988) mentioned that the cytological analysis of isolated germ cells showed different features from those observed in histological sections.

To eliminate the possibility that some seminiferous tubules might exhibit more active spermatogenesis than others (Berndtson, 1977), moving of the fine needle within the testis to collect cells from larger areas was performed. In this respect, Gottschalk-Sabag et al., (1995) reported that more than one testicular specimens were needed to evaluate the spermatogenic process quantitatively.

In the current study, the quantitative analysis of cytological smears of apparently normal stallions (86.7%) revealed that there was a high percentage of spermatids and spermatic index and low Sertoli index. Based on clinical and cytologial bases, these stallions appeared to be normozoospermic. Spermatid and spermatozoa were a valuable parameters when searching for disturbed spermiogenesis. As variation in spermatogenesis, at any stage, should also be reflected in corresponding changes in the spermatid number (Berndtson, 1977; Johnson, 1991).

Regarding the affected animals, there was significant decrease in the mean values of primary spermatocytes, secondary spermatocytes, early spermatids and spermatic index. The Sertoli index showed a high significant increase. These animals (23.3%) appeared to be oligo or azoo-spermic. In this respect, the ratio of Sertoli cells to spermatogenic cells (Sertoli cells to spermatogenic cells (Sertoli index) constituted an attempt to simplify the cytological results because the number of Sertoli cells is constant per unit of tubular length (Berndtson, 1977). Morecver, an increase in the Sertoli index may reveal the decrease in the tubular germ cells population. Testicular fibrosis resulted in shortening of the tubules and hence a loss of Sertoli cells. In these circumstances, the Sertoli index may not be stable and spermiogenesis will be revealed by an associated reduction of the spermatozoa percentage (Johnson, 1991; Johnson and Tatum, 1991).

The histopathological investigations of apparently normal animals revealed intact seminiferous tubules with the presence of all types of germ cells while the affected stallions, showed degenerative changes in the seminiferous tubules. As reported by Watson, et, al., (1994); Watson, (1998) seminiferous tubules were sensitive to a variety of physical, chemical, endocrine and pathologic disturbances which may lead to different forms of testicular degeneration and needs an acurrate methods of diagnosis. In the present work, the histopathological investigation confirmed the

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results obtained by fine needle aspiration cytology of normal and affected stallions.

In conclusion, fine needle aspiration cytology is non-invasive, reproducible technique and can provide adequate diagnostic cytological material for qualitative and quantitative analysis of testicular affections in stallions.

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