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Semen Quality and Chromatin Condensation in Domestic Cat Sperm During Passage Through the Epididymis

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ABSTRACT

The objectives were to identify and quantify changes occurring in cat sperm as they pass through the epididymis, vas deferens and ejaculate. Cat epididymides obtained by orchidectomy were divided into six regions (1 to 6) and sperm cells were released by mincing each epididymal region. The semen quality was assessed by light microscopy and the sperm chromatin condensation was measured by flow cytometry. The sperm motility was minimal in region 1 and increased significantly ($p < 0.05$) when the sperm passed from this region, with maximum motility reached in region 6, the vas deferens and ejaculate. Progressive motility and the percentage of sperm with normal morphology increased during epididymal transit. The most prevalent defects in abnormal spermatozoa were found in the tail. The mean degree of maturation (± standard error of the mean) of sperm chromatin condensation was 72.9 ± 9.4% in region 1 and was significantly ($p < 0.05$) increased in region 3 (94.4 ± 2.6%). Chromatin condensation or decondensation processes were studied further in cat epididymides (four portions: initial segment, caput, corpus and cauda) by incubating sperm with alkaline phosphatase (AP) or dithiothreitol (DTT), respectively, followed by staining with propidium iodide and analysis by flow cytometry. After AP treatment, the fluorescence intensity approximated that found in the initial segment. The DTT treatment significantly ($p < 0.05$) increased the fluorescence of the chromatin condensation in all parts, except the initial segment. Cat sperm chromatin was shown to undergo condensation during passage through the epididymis and the condensation process was reversed by reducing disulfide to sulfhydryl bonds or promoted by dephosphorylation.

Keywords: chromatin condensation, domestic cat, epididymis, sperm

INTRODUCTION

The domestic cat (*Felis catus*) is commonly used as a valuable animal model for comparative reproductive studies of endangered felids, such as the tiger (*Panthera tigris*), clouded leopard (*Neofelis nebulosa*), flat-headed cat (*Prionailurus planiceps*), marble cat (*Pardofelis marmmorata*) and leopard (*Panthera pardus*). These felids share many characteristics in reproductive performance and therefore, the present study on semen quality and sperm

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chromatin condensation during passage through the epididymis of the domestic cat provides basic knowledge for understanding the male reproductive physiology in felid species.

During the process of spermiogenesis, the testicular haploid cells undergo extensive differentiation, which results in a change of form to the characteristic spermatozoa shape, with a flagellar tail structure, a compact nucleus containing condensed chromatin, and acrosomal organelles. Sperm cells that leave the testis are not capable of normal motility and fertility. These abilities are acquired during their transit through the epididymis, where the spermatozoa undergo various morphological and functional changes (Franca *et al.,* 2005). Studies on chromatin condensation in several mammalian species demonstrated that sperm chromatin condensation starts during sperm production in the testis but it was not complete. Further condensation occurs by oxidation of the thiol group of cysteine residues to disulfides, as they pass through the epididymal lumen (Perreault *et al.,* 1987). During spermiogenesis, transitional proteins gradually replace somatic cell-like histone proteins. Protamines, which are rich in thiol (sulfhydryl) groups, ultimately replace the transitional proteins. Balhorn (1982) proposed that these protamines bind to the DNA by lying lengthwise along the minor groove of the double stranded molecule. Regardless of specific groove placement, these protamines contain enough positively charged arginine groups on the DNA strand, so that the amino- and carboxy-terminal ends of the protamine molecule are folded inward toward the center of the molecule and are locked into place, each by a single intramolecular disulfide bond (Balhorn *et al.,* 1991). During transport through the epididymis, the thiol groups undergo oxidation resulting in the formation of many disulfide groups (S-S) that give the chromatin its high degree of compaction and stability in ejaculated sperm.

The chromatin condensation in the sperm head can be studied by using the fluorescent probes, acridine orange (Evenson, 1990) and propidium iodide (Krishnamurthy *et al.,* 2000), to stain DNA by intercalating between the bases in the double helix. Acridine orange is a metachromatic dye and fluoresces green when bound to double stranded DNA, but is a red color when bound to single stranded DNA (Ichimura *et al.,* 1971), whereas, propidium iodide produces a red fluorescence when bound to double stranded DNA. A previous study in cat epididymal spermatozoa showed that the percentage of sperm with stable double stranded DNA (acridine orange staining) increased significantly from caput to corpus and cauda, respectively (Hingst *et al.,* 1995). However that study used fluorescence microscopy that is laborious and time consuming and only 200 sperm nuclei were examined. The use of flow cytometry to measure the chromatin condensation process following fluorescent staining provides an objective and more accurate method than conventional microscopy, since several thousand sperm cells can be analyzed in a few seconds. Sperm chromatin condensation using flow cytometric analysis has been reported in several mammalian species (Yossefi *et al.,* 1994; Golan *et al.,* 1996, 1997; Lewin *et al.,* 1999). Flow cytometric analysis has also been used to study perm chromatin condensation in the felidae (Penfold *et al.,* 2003; Neubauer *et al.,* 2004; Blottner and Jewgenow, 2007; Siemieniuch and Dubiel, 2007). The objectives of the present study were to identify and quantify: 1) the domestic cat sperm motility and morphology in various regions of the epididymis, vas deferens and in the ejaculate; 2) the sperm chromatin condensation of the domestic cat spermatozoa during transit through the epididymis using flow cytometric assay; and 3) the effects of reduction of disulfide groups and of dephosphorylation by alkaline phosphatase on maturation of chromatin condensation of spermatozoa from various loci in the epididymis.

MATERIALS AND METHODS

Chemicals

All chemicals in this study were purchased from the Sigma Chemical Company (Sigma, St. Louis, MO, USA), unless stated otherwise.

Animal and semen collections

Postpubertal privately owned male domestic cats (aged 1-3 y), submitted for routine surgical orchidectomy were used for semen collection. In this study, semen was collected by electroejaculation before orchidectomy under anesthesia. Ejaculated semen was diluted with 37°C Ham's F-10 medium containing 10% fetal calf serum. The epididymis and vas deferens were removed within 1 h after orchidectomy. The epididymis was dissected free from the testis in normal saline solution, inspected macroscopically and divided into six regions (Axnér *et al.,* 1999). Spermatozoa were released by mincing each epididymal region and the vas deferens with fine scissors in 1 mL of 37°C Ham's F-10 medium. The sperm suspension was filtered through 41 μm nylon mesh in order to discard tissue remnants and debris. A sample (20 μL) of the sperm suspension was taken for motility and morphology analysis and the remainder was frozen at -80°C in TNE buffer (0.01 M Tris buffer, 0.15 M NaCl, 1 mM EDTA, pH 7.4) supplemented with 10% glycerol for flow cytometric analysis.

Semen analysis

The methods used for semen analysis followed procedures of the World Health Organization laboratory manual (WHO, 1992). A Makler counting chamber (Sefi-Medical Instruments Ltd., Haifa, Israel) was used to assess the percentage of progressively motile sperm after dilution with Ham's F-10 medium at 37°C. For morphological analysis, an aliquot of 10 μL of the sample was spread onto a microscopic slide and stained with Giemsa stain (Diff-Quik, Baxter Scientific, McGaw Park, IL, USA). Morphology assessment was performed under a light microscope at ×1,000 magnification under oil immersion. At least 200 spermatozoa were counted randomly on each slide.

FACS analysis of chromatin condensation using acridine orange staining

The method for acridine orange staining and flow cytometry of spermatozoa followed the two step procedure of Evenson (1990). The frozen spermatozoa suspension was thawed and centrifuged at 9,000 rpm for 5 min. The sperm pellet was resuspended in 100 μL TNE buffer. The suspension was subjected to brief acid denaturation by mixing with 200 μL of chilled lysis solution (0.1% Triton X-100 [v/v], 0.15 M NaCl, 0.08 M HCl, pH 1.4), held for 30 sec, and mixed with 600 μL of acridine orange (Molecular Probes, Eugene, OR, USA) solution (6 μg acridine orange/mL in buffer: 0.037 M citric acid, 0.126 M Na2HPO4, 1.1 mM EDTA, 0.15 M NaCl, pH 6). After at least 3 min, the chilled sample was aspirated into a FACS Vantage flow cytometer (Becton Dickinson; San Jose, CA, USA) using FACS flow as the sheath fluid. The flow cytometer was standardized for each analysis session by using standard beads (Calibrite Beads, Becton Dickinson). Sperm cells were analyzed for green or red fluorescence emission at a rate of 500–1000 cells/s and 10,000 events for each sample were recorded for further analysis with the WinMDI computer software, version 2.8 (Trotter, 2000).

Measurement of maturation of chromatin condensation

The measurement of maturation of chromatin condensation was carried out as described previously (Golan *et al.,* 1996). A maturation zone (window M; WINM) which contained the bulk of sperm nuclei obtained from 10,000 cells of the ejaculated spermatozoa was

drawn on a scattergram of red versus green fluorescence (Figure 1). Another zone (window T; WINT), was designed to include the major sperm band, excluding material with very low fluorescence (cell debris) and also excluding cells containing abnormally high red:green fluorescence ratios. The percent maturation was calculated using Equation 1:

$$\% \text{ maturation} = 100 \times \frac{\text{WINM}}{\text{WINT}} \qquad (1)$$

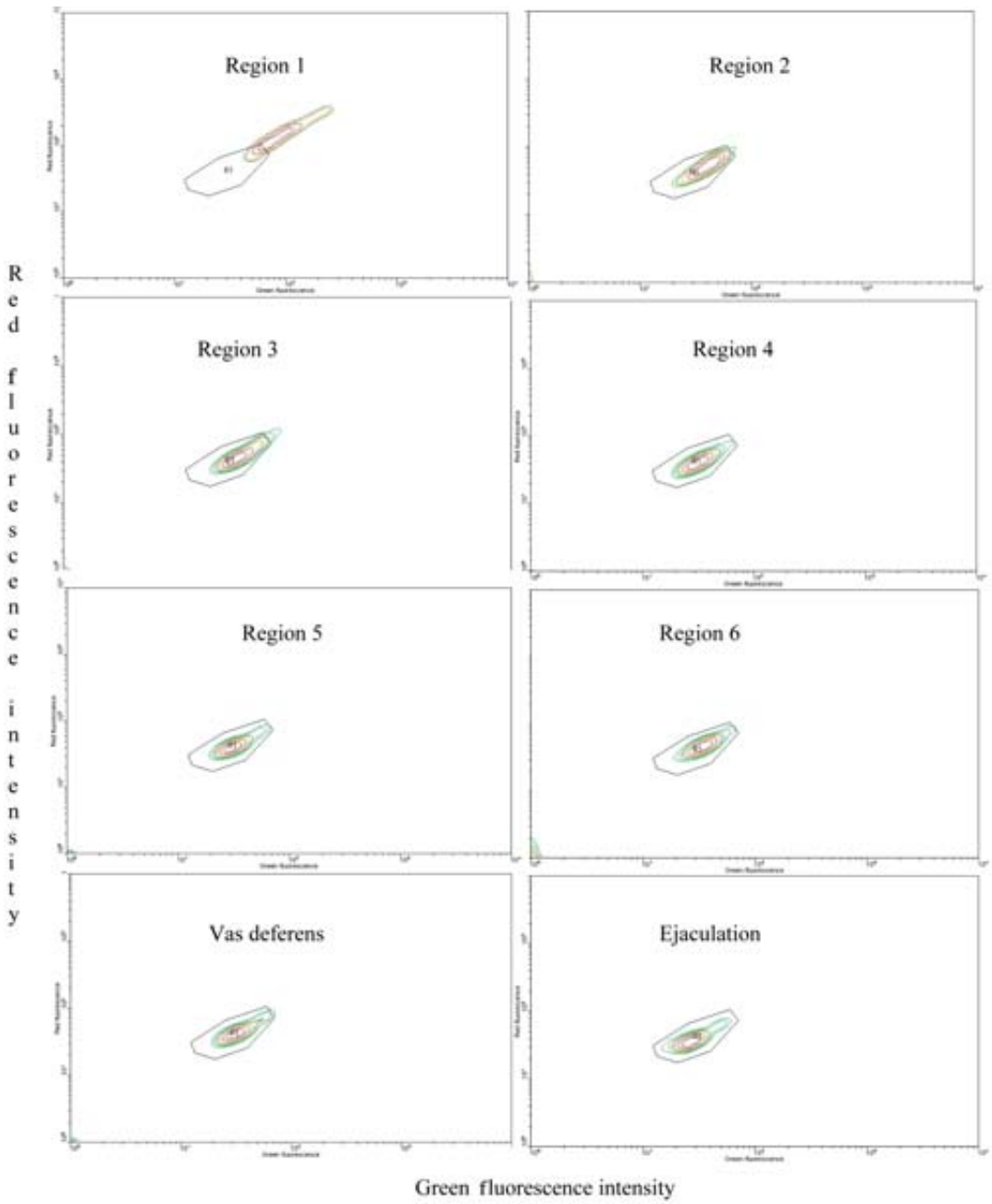

Figure 1 Scattergram of cat spermatozoa stained with acridine orange and obtained from various regions of the epididymis, vas deferens and ejaculate.

Dithiothreitol (DTT) and alkaline phosphatase (AP) treatments

In order to collect enough sperm to study the effects of disulfide reduction and of dephosphorylation on the chromatin condensation process, the epididymis was divided into four portions: initial segment, caput, corpus and cauda, and the spermatozoa were collected as described above. The frozen spermatozoa suspension was thawed and centrifuged at 9,000 rpm for 5 min. The sperm pellet was resuspended either with 100 µL of 25 mM DTT or 40 mg/mL AP in TNE buffer containing 0.1% Triton X-100 (v/v) and then incubated at 37°C for 30 min. At the end of incubation, the sperm suspension was centrifuged at 9,000 rpm for 5 min and the sperm pellet was resuspended in 500 µL TNE buffer containing 0.5 mg/mL RNase and 0.1% Triton X-100 (v/v) and was incubated for 30 min at 37°C. At the end of incubation, the solution was mixed with 500 µL cold propidium iodide (PI) solution [TNE buffer containing 25 µg/mL PI and 0.1% Triton X-100 (v/v) in final concentration]. The solution was incubated on ice for 30 min and aspirated into a flow cytometer. Sperm cells were analyzed for red fluorescence emission at a rate of 500-1000 cells/s and 10,000 events for each sample were recorded for further analysis with the computer software WinMDI, version 2.8 (Trotter, 2000).

Statistical analysis

All data were expressed as (mean ± standard error of the mean). The difference in sperm morphology, motility and sperm chromatin condensation among various regions of the epididymis, vas deferens and ejaculate and the peak values following treatment with or without DTT and AP treatments were analyzed using ANOVA; when necessary, differences were determined with Duncan's new multiple range test. The analyses were carried out with the SPSS 9.0 computer software (SPSS Inc., Chicago, IL, USA) and the level $p < 0.05$ was considered significant.

RESULTS

Sperm motility

Sperm motility of cat spermatozoa obtained from various regions of the epididymis, vas deferens and ejaculation is shown in Table 1. Little progressive motility of spermatozoa was found in region 1 of the epididymis and the motility increased significantly ($p < 0.05$) as spermatozoa passed through more distal epididymal regions, then into the vas deferens and ejaculate. The most pronounced overall percent motility was seen in region 6, the vas deferens and ejaculate.

Table 1 Percentage of progressive motility of cat spermatozoa obtained from various regions of the epididymis, vas deferens and ejaculate.

	Region	n	% Motility
Epididymis	1	22	2.23 ± 0.50^{a}
	2	22	6.32 ± 1.20^{b}
	3	22	16.05 ± 2.13^{c}
	4	22	33.32 ± 3.50^{d}
	5	22	57.82 ± 2.26^{e}
	6	22	67.82 ± 2.39^{f}
Vas deferens	-	13	63.46 ± 3.41^{f}
Ejaculate	-	10	68.00 ± 2.60^{f}

Values shown are mean ± standard error of the mean; n = number of animals; $^{a-f}$ = different superscripts within a column indicate a significant difference ($P < 0.05$).

Sperm morphology

The percentage of spermatozoa with normal morphology was 64.9 ± 0.6 and 66.5 ± 0.9 in regions 1 and 2, respectively and increased significantly ($p < 0.05$) as spermatozoa moved from region 3 through region 6 and into the vas deferens and ejaculate (Table 2). Spermatozoa with abnormal heads accounted for less than 4% of the total. The head abnormalities were classified as macrocephalic, microcephalic, bicephalic and tricephalic types. All of these abnormalities decreased progressively from region 3 through region 6, the vas deferens and ejaculate (data not shown). There were more cells with abnormalities in the tail than in the head. The percentages of sperm tail abnormalities were similar in regions 1 and 2 and decreased progressively from region 3 through region 6 and in the vas deferens and ejaculate (Table 2). The abnormalities of the sperm tail were classified as coiled tail, bent tail, two tails, detached head, and proximal and distal cytoplasmic droplets. The percentages of spermatozoa with coiled tail decreased significantly ($p < 0.05$) in region 3, 4 and 5. On the contrary, the percentages of bent tail spermatozoa increased significantly ($p < 0.05$) in region 3 and 5 and decreased significantly ($p < 0.05$) in the vas deferens. There was no difference in the number of two-tailed spermatozoa in ejaculate, the vas deferens and various regions of the epididymis. The percentage of spermatozoa with detached heads decreased significantly ($p < 0.05$) in region 3 and in ejaculate. The presence of proximal cytoplasmic droplets on spermatozoa decreased significantly ($p < 0.05$) when they reached regions 3, 4 and 5. The highest proportion of spermatozoa bearing distal cytoplasmic droplets was found in region 3 and decreased significantly ($p < 0.05$) when the spermatozoa passed through region 5.

FACS analysis of chromatin condensation using acridine orange staining

The results of flow cytometry demonstrated that both green and red fluorescence were emitted by all cells, but at different intensities, depending upon the accessibility of the dye to the DNA. The scattergrams of acridine-orange-stained spermatozoa obtained from ejaculate, the vas deferens and various regions of the epididymis are shown in Figure 1. As seen in the scattergram, the fluorescence of chromatin in spermatozoa decreased from region 1 of the epididymis to the ejaculate.

Maturation of chromatin condensation

The maturation of chromatin condensation in cat spermatozoa obtained from

Table 2 Percentage of normal and abnormal spermatozoa obtained from various regions of the epididymis, vas deferens and ejaculate.

	Region	n	Normal morphology	Abnormal head	Abnormal tail
	1	15	64.9 ± 0.6^{a}	3.1 ± 0.3^{a}	29.5 ± 2.4^{a}
	2	15	66.5 ± 0.9^{a}	2.2 ± 0.3^{b}	28.9 ± 2.5^{a}
	3	15	70.3 ± 1.1^{b}	1.1 ± 0.2^{c}	26.1 ± 2.3^{b}
Epididymis	4	15	71.8 ± 0.7^{b}	1.4 ± 0.2^{c}	26.6 ± 0.7^{b}
	5	15	72.5 ± 0.4^{b}	1.1 ± 0.2^{c}	26.3 ± 0.4^{b}
	6	15	$7\ 3.0 \pm 0.5^{b}$	1.4 ± 0.2^{c}	25.9 ± 0.5^{b}
Vas deferens	-	10	74.5 ± 1.0^{c}	$2.3 \pm 0.2^{b,d}$	23.3 ± 0.8^{c}
Ejaculation	-	7	76.9 ± 0.6^{d}	$1.6 \pm 0.2^{c,e}$	21.6 ± 0.5^{c}

Values shown are mean ± standard error of the mean; n=number of animals; ^{a,b,c} = different superscripts within a column indicate a significant difference ($P < 0.05$).

ejaculate, the vas deferens and various regions of the epididymis is shown in Table 3 and Figure 1. There was no significant ($p < 0.05$) difference between the percentage of chromatin condensation maturation in regions 1 and 2. The first significant ($p < 0.05$) increase of maturation occurred in region 3. Finally, the maturation of chromatin was highest in spermatozoa obtained from region 5 and 6 of the epididymis, vas deferens and ejaculate.

Dithiothreitol and alkaline phosphatase treatments

Epididymal spermatozoa after DTT treatment showed significant ($p < 0.05$) increases of fluorescence intensity in caput, corpus and cauda compared with the untreated cells (Figure 2). A smaller increase, which was not statistically significant, was seen in the initial segment. The differences in fluorescence intensity between the control and DTT-treated sperm increased progressively from the initial segment to caput and corpus, and were greatest in the cauda epididymal sperm. The reverse effect was seen in the study using the AP treatment (Figure 3). Here, the differences in fluorescence intensity between the control and AP-treated sperm decreased progressively from the initial segment to caput and corpus, and was least in the cauda epididymal sperm. This provided evidence that dephosphorylation takes place during passage of sperm through the epididymis.

DISCUSSION

The present study produced values of some parameters of samples of spermatozoa obtained during the transit of spermatozoa through the epididymis of domestic cats. These included the percent of progressive motility in cells obtained from different locations in the epididymis, in the vas deferens and in ejaculate samples, the types and amounts of morphological defects found in these locations and the degree of maturation of chromatin condensation found there. An investigation of the effects of incubation of the sperm with dithiothreitol or with alkaline phosphatase provides evidence on two of the reactions involved in chromatin condensation. Due to difficulties in macroscopically defining where the regions of the epididymis began and ended, and since the coiling of the epididymal duct made it impossible to cut the duct exactly between regions, some of the spermatozoa may have been from the neighboring regions.

The greatest amount of morphological sperm abnormalities in the domestic cats sampled were found in the proximal caput (region 1, 2) and a significant reduction in the proportion of

Table 3 Maturation of chromatin condensation in cat spermatozoa obtained from various regions of the epididymis, vas deferens and ejaculate.

	Region	N	Maturation (%)
	1	5	72.9 ± 9.4[a]
	2	7	9.9 ± 10.9[a,b]
	3	7	94.4 ± 2.6[b]
Epididymis	4	7	97.7 ± 0.9[b]
	5	7	99.5 ± 0.1[c]
	6	7	99.2 ± 0.5[c]
Vas deferens	-	7	98.8 ± 0.8[c]
Ejaculation	-	7	98.9 ± 0.3[c]

Values shown are mean ± standard error of the mean; n = number of animals; [a,b,c] = different superscripts within a column indicate a significant difference ($P < 0.05$).

spermatozoa with abnormalities occurred during epididymal transit. This suggested that the epididymis may play a role in removing defective spermatozoa during their passage through it. Spermatozoa with tail abnormalities were more common than abnormalities in the head and most of the defects were bent midpieces or cytoplasmic droplets on the tail. The high percentage of abnormal sperm tails found in the epididymis in the present study was higher than those described previously (Axnér *et al.,* 1998). The discrepancy may have been due to differences in the criteria used for classification of an abnormal sperm tail, since coiled tail, bent tail, two tails, detached head, proximal and distal cytoplasmic droplets were classified as abnormalities of the sperm tail in the present study, whereas, only single bent tail, coiled tails and double bent tails were classified as abnormalities of the sperm tail in the study by Axnér *et al.* (1998). The percentage of spermatozoa with abnormal heads and tails decreased significantly from region 1 to the

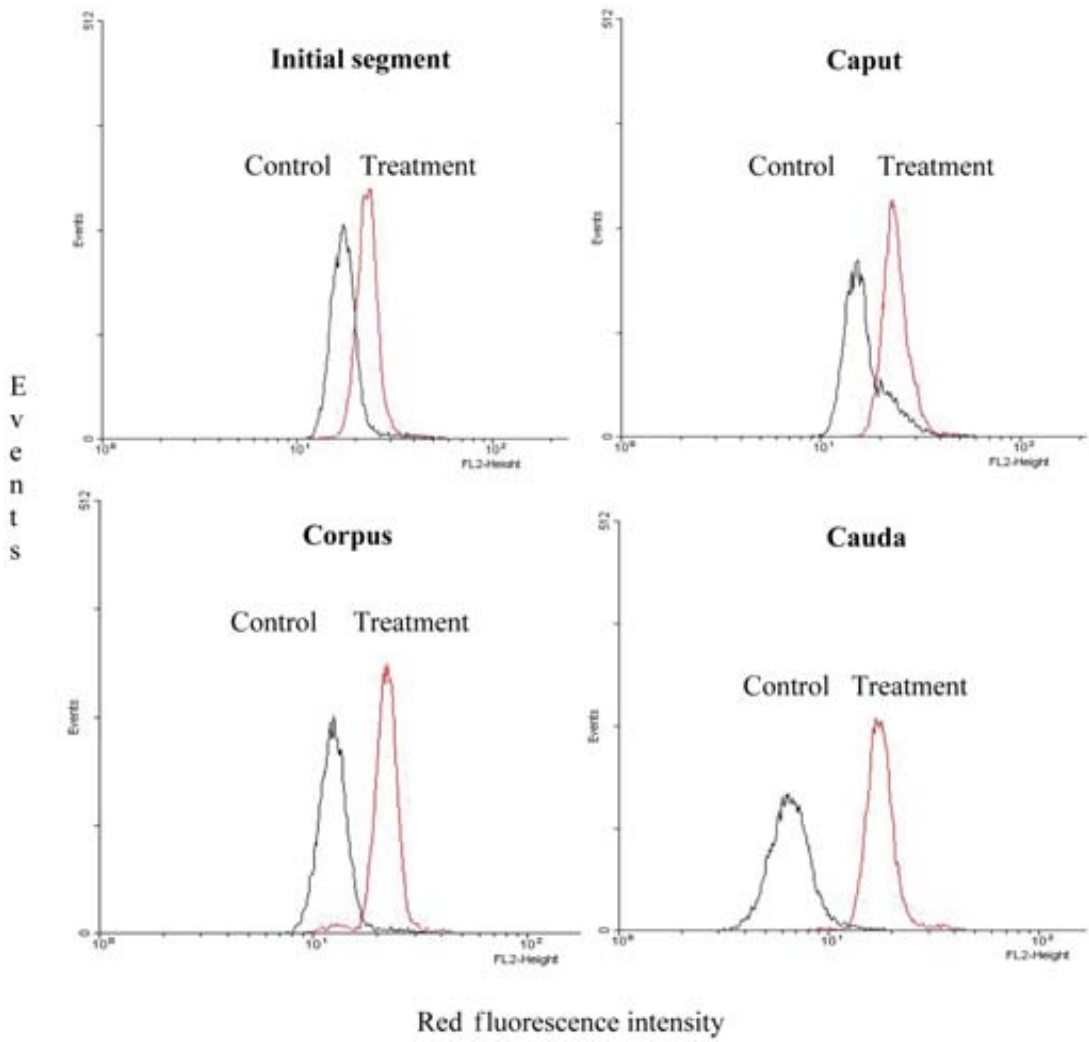

Figure 2 Comparison of control histograms of cat spermatozoa stained with propidium iodide and obtained from four regions of the epididymis, with similar samples that had been treated with dithiothreitol.

ejaculate, except for spermatozoa with bent tails or distal cytoplasmic droplets, which both increased in the distal regions of the epididymis. The present results confirmed a previous study that the proportion of spermatozoa with abnormalities of the tail increased as spermatozoa traversed the epididymis (Axnér *et al.,* 1999). In a different animal species, such as boar (Briz *et al.,* 1996), the frequency of abnormalities of the sperm tail showed the same effect. Studies in bulls (Blom and Wolstrup, 1976) and humans (Eliasson and Lindholm, 1974) suggested that abnormality of the tail was closely related with a weakness in the outer dense fibers caused by a zinc excess in the epididymal fluid. The zinc excess during epididymal maturation prevents the formation of disulfide cross-links between cysteines of the dense fibers, resulting in a weakness of these fibers

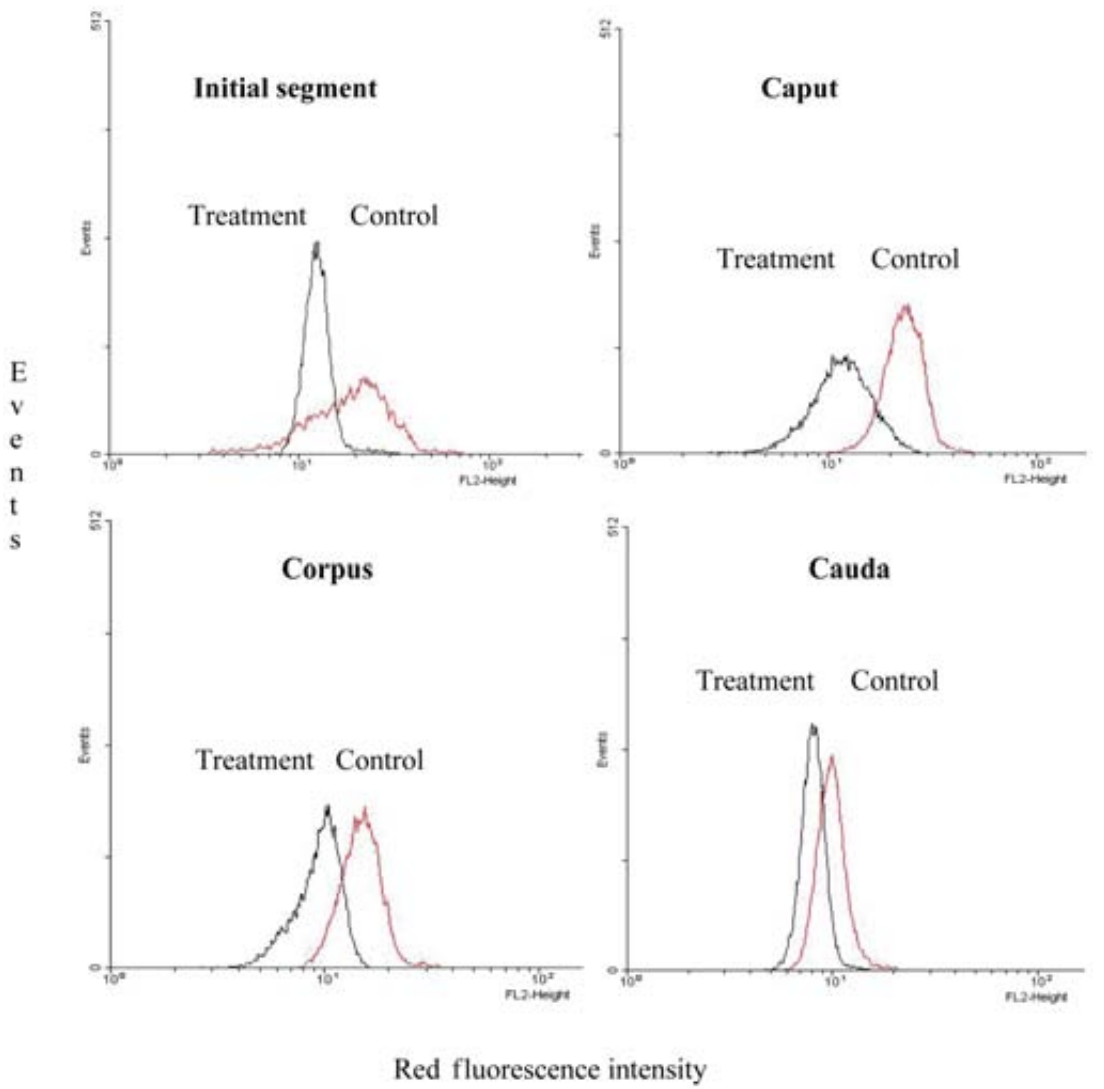

Figure 3 Comparison of control histograms of cat spermatozoa stained with propidium iodide and obtained from four regions of the epididymis, with similar samples that had been treated with alkaline phosphatase.

and consequently yielding spermatozoa with folded or coiled tails. The incidence of specific types of sperm abnormalities appears to be species-specific. Cheetah, leopard and puma ejaculates contain many spermatozoa with tightly coiled tails or bent tails, whereas lion semen contains spermatozoa with a higher incidence of cytoplasmic droplets and flagellar bending (Wildt *et al.,* 1983, 1988). However, the precise etiology of specific pleiomorphic spermatozoa in the felids is unknown.

The progressive migration of the cytoplasmic droplet along the midpiece of the spermatozoa and its loss as it reaches the more distal sites of the epididymis also contributes to defining some defects in morphology. For most spermatozoa, this migration occurred in region 3. The location of the cytoplasmic droplet varies considerably between species. Whereas in rabbits (Perez-Sanchez *et al.,* 1997), this takes place fundamentally in the transition from the proximal to distal caput of the epididymis, in rats, most of the spermatozoa in the caput of the epididymis already had distal droplets (Robaire and Hermo, 1988). Considering the data obtained from the present study, the movement of the cytoplasmic droplet in the domestic cat did not take place until the spermatozoa had reached region 3. In the ram and boar, the movement of the cytoplasmic droplet preceded the appearance of the first motile spermatozoa (Amann *et al.,* 1982; Dacheux *et al.,* 1989). The simultaneous occurrence of cytoplasmic droplet movement and the onset of sperm motility, indicate that important changes in the maturation of spermatozoa take place in region 3 of the cat epididymis.

The reduced access of acridine orange to the DNA has been used to measure tightness of DNA-protein binding in sperm chromatin (Evenson, 1990). This reflects the process of protamine binding to the minor groove of DNA (Ward and Coffey, 1991). This characteristic reflects the process of chromatin packaging that is essential for spermatozoa to protect their genetic material from physical and chemical damage and to streamline the head to reduce resistance during motility. Chromatin condensation in cat epididymal spermatozoa has been reported using aniline blue and acridine orange and observed under conventional and fluorescent microscopes (Hingst *et al.,* 1995). They found that chromatin stability increased significantly from the caput epididymal region to the cauda epididymal region. The present study using flow cytometry showed that the maturation of chromatin condensation in cat spermatozoa in region 1 of the epididymis was 72.9% and further condensation occurred as they passed through each region of the epididymis. It is interesting to note that the maturation of chromatin condensation in cat epididymal spermatozoa observed in the present study was higher than that observed in other species. Golan *et al.* (1996) demonstrated that the maturation of chromatin in human spermatozoa was 28% in the caput epididymis and was 70% when the spermatozoa had reached the distal cauda epididymis. Similarly, a study in hamster spermatozoa showed that the maturation of spermatozoa was 24% and 94% in the proximal caput and cauda epididymis, respectively (Yossefi *et al.,* 1994).

The present investigation used propidium iodide to study the effects of incubation with DTT or AP on the degree of chromatin condensation of epididymal spermatozoa. Mammalian protamines are characterized by the presence of arginine and cysteine residues (Balhorn, 1982). Dephosphorylation and phosphorylation have been shown to play a role in chromatin condensation and decondensation in sperm nuclei (Pruslin *et al.,* 1987). Sperm nuclear histones are more highly phosphorylated in the testis than the epididymis. Therefore the caput spermatozoa might contain phosphate groups whose negative charges would distance the protamine from DNA. Protein dephosphorylation might increase the tightness of

fit between DNA and protamines, thus effecting chromatin condensation. To test this hypothesis, alkaline phosphatase was employed. The result demonstrated that the effect of alkaline phosphatase treatment was found to a greater degree in sperm from the initial segment than in those obtained from more distal sites. The effect of alkaline phosphatase treatment occurred in all parts of the epididymis, except in the cauda epididymis. This suggests that a portion of the sperm chromatin condensation that occurs in the proximal caput epididymis might be a consequence of dephosphorylation taking place there and that the natural dephosphorylation process was complete by the time the sperm reached the cauda epididymis. A similar conclusion had been reached by Yossefi *et al*. (1994) using hamster spermatozoa.

The importance of oxidation of protamine sulfhydryl to disulfide groups in the chromatin condensation process has been reported (Bedford and Calvin, 1974). The locations in the epididymis where sulfhydryl oxidation occurred were studied by incubation with DTT, in order to reduce disulfide bonds to their sulfhydryl state. The results showed that oxidation of cat sperm sulfhydryl to disulfide groups occurred mostly in the distal epididymal regions, whereas a considerable degree of chromatin condensation had occurred during the passage of sperm through the initial segment of the epididymis before oxidation of any significant percentage of the sulfhydryl groups had taken place. This was consistent with the report of Yossefi *et al*. (1994) that the degree of oxidation of sulfhydryl groups to disulfides in hamster sperm nuclei was less in caput epididymal spermatozoa than in the distal epididymal spermatozoa. DTT treatment that reduced disulfides to sulfhydryl groups moved cauda epididymal sperm nuclei out of the zone characteristic for chromatin-condensed nuclei.

In conclusion, motility and morphological maturation of cat spermatozoa increased during epididymal transit. The cat sperm chromatin underwent condensation during its passage through the epididymis and the condensation process can be reversed by reducing disulfide to sulfhydryl bonds or promoted by removing phosphate groups. These findings in the domestic cat may be important for understanding male reproductive physiology in other endangered felid species.

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