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RESEARCH ARTICLES

Effect of Cetrorelix on Sperm Morphology During Migration Through the Epididymis in the Cynomolgus Macaque (*Macaca fascicularis*)

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The importance of the cynomolgus monkey as a model for human reproductive medicine prompted this examination of epididymal sperm morphology. Computer-aided sperm morphological analysis was used for the first time to provide morphometric data on sperm heads as they traversed the epididymal duct of *Macaca fascicularis*. The duct was divided into six regions, starting close to the testis (proximal) and ending close to the vas deferens (distal). To determine the androgen-dependence of the changes, one group of animals received a GnRH-antagonist (Cetrorelix, Asta Medica, Frankfurt, Germany) to induce testicular regression and lower epididymal androgens, while a control group received only vehicle. Epididymides were removed 16 and 25 days after treatment, and sperm heads were analysed by a computer-assisted morphometric analyser. Cluster analysis revealed swollen sperm head cells in proximal regions 1 and 2 of the epididymis, but fewer such forms distally. Normal head shapes became the majority in region 4 and these underwent a gradual but statistically significant decrease in size (area, perimeter, length, width) and shape as they reached the distal regions. In the animals given Cetrorelix, sperm with swollen heads were found more distally than in the controls, although they were also never present in the distal cauda (region 6). Normal heads still became predominant in region 4 after 16 days treatment, and in region 6 after 25 days. The normal forms in the cauda epididymidis of treated animals were significantly larger than cells from control animals. We conclude that epididymal sperm maturation in the monkey is characterised by both a loss of sensitivity to distortion on air-drying, and by a decrease in sperm head size. The former, but not the latter, is attained by sperm in androgen-deficient epididymides from GnRH-antagonist-treated monkeys. Am. J. Primatol. 51:103–117, 2000. © 2000 Wiley-Liss, Inc.

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INTRODUCTION

Cynomolgus monkeys are increasingly used as animal models for human reproductive toxicology [Korte, 1999] and in the development of an endocrine contraceptive for males [Nieschlag et al., 1999]. For such contraceptive agents inhibiting spermatogenesis, azoospermia is anticipated, although abnormal sperm forms may be present as the drug takes effect. In contrast, post-testicular approaches to contraception [Cooper & Yeung, 1999a] depend on damage to sperm in the epididymis. In both cases morphological examination of spermatozoa in the ejaculate could be informative as to the fertility status of the male. The morphology of ejaculated spermatozoa reflects the state of the testis that produced them as well as that of the epididymis, which is responsible for post-testicular changes. Various aspects of the epididymal maturation of spermatozoa of non-human primates resemble those in man [Cooper & Yeung, 1999b], but the morphology has not been examined objectively. One of the first reported features of epididymal sperm maturation was the gross morphological change in the nucleus and acrosome of the guinea pig, which was subsequently shown to be androgen dependent [Blaquier et al., 1972]. While this is an obvious change, less spectacular changes also occur in the sperm of other species. (To date, however, only a few morphometric studies have been conducted on rodents [De Rosas & Burgos, 1987; Fornés & De Rosas, 1989].) In contrast, primate sperm do not exhibit any such obvious changes upon maturation and the few descriptive reports on the changes in sperm head morphology during epididymal maturation in monkeys were made by electron microscopy [Bedford & Nicander, 1971; Moore et al., 1984].

Recently, attempts to inject objectivity into sperm morphology assessment has resulted in image analysis systems being marketed that automate the evaluation of sperm morphology and hold promise of rapid and unbiased assessment of sperm morphology for human ejaculates [Davis et al., 1992; Garrett & Baker, 1995; de Monserrat et al., 1995; Hofmann et al., 1996; Kruger et al., 1996]. One of these systems has been used to provide a detailed analysis of sperm head morphometry during epididymal passage in the rabbit [Pérez-Sánchez et al., 1998] and mouse [Tablado, 1996]. This equipment was used in the present study to determine the morphological changes that normally occur in sperm as they pass through the non-human primate epididymis. In addition, the effects of androgens on this process were examined by administering a GnRH-antagonist (Cetrorelix) that reduces both testicular and epididymal androgens [Yeung et al., 1999] before spermatogenic arrest depletes the epididymis of sperm. This treatment leads to changes in the normal maturation of sperm with respect to chromatin condensation [Golan et al., 1997] and acquisition of motility [Yeung et al., 1999]. In the latter work, subjective changes in sperm head morphology due to this treatment were observed, and these are examined here in more detail.

METHODS

Treatment of Animals

Monkeys used in the study of Weinbauer et al [1998] were used. Ten healthy crab-eating monkeys (*Macaca fascicularis*) with a mean (± sem) body weight of 5.8 ± 0.3 kg were used. In order to examine the androgen-dependence of male reproductive function in the presence of the testes, males were treated with a gonadotrophin-releasing hormone antagonist (Cetrorelix), that depresses pituitary release of LH and FSH. Lowered LH in turn depresses the testicular secretion of testosterone, which is essential for some aspects of epididymal function.

Males were divided into two groups of five, one group receiving Cetrorelix (450 μg/kg for up to 25 days dissolved in vehicle) by subcutaneous injection, and the other receiving vehicle (glucose 5.25%, w/v, in saline). Cetrorelix was a generous gift from Dr. T. Reissmann, Asta Medica, Frankfurt. This treatment reduced testicular volume 20% at 16 days and 35% by 25 days, serum testosterone from about 20 to 2 nmol/l, and epididymal androgens about 50% at both time points [Yeung et al., 1999].

Animals were caged individually in a temperature- and humidity-controlled environment with a 12L:12D cycle and had regular access to food and water. The males were examined after the injection at times dictated by the anticipated incorporation of bromo-deoxyuridine into S-phase cells of the testis: 16 days after the initial injection, when the animals were hemi-castrated, and again after 25 days, when the remaining testis and epididymis were removed from the antagonist-treated males [Weinbauer et al., 1998]. Animals were anesthetized for the operations with 12 mg/kg ketamine (Parke-Davis, Munich, Germany) and anesthesia was maintained by intravenous injection of 6 mg/kg sodium pentobarbitone (Nembutal; CEVA, Paris, France).

Collection of Epididymal Spermatozoa

Spermatozoa were obtained from 10 monkeys, from six regions of the epididymides: 1) the initial segment; 2) the caput region, where the tubule was more darkly pigmented than adjacent regions; 3) the proximal corpus; 4) the mid-corpus; 5) the proximal cauda; and 6) the distal cauda epididymidis (Fig. 1). This was accomplished by excising 2–4 mm lengths of tubule and mincing them in a small volume of phosphate-buffered saline (PBS; Gibco, Eggenstein, Germany) in a Petri dish that was shaken to release the spermatozoa. Smears were made from these sperm suspensions, air-dried and fixed [WHO, 1992] and stained with Hemacolor™ (Merck, Cat. No. 1161, Darmstadt, Germany). Slides were evaluated by the Sperm-Class Analyzer® (Microptic, Barcelona, Spain) (see below).

Evaluation of Artifacts Production

Preparations of spermatozoa, released into PBS from regions 1 to 6 of the epididymis from one control monkey, were either smeared on slides, air-dried and fixed [WHO, 1992] or fixed first with 80% (v/v) ethanol before smearing and air drying. Ethanol was used as the fixative as it comprises part of the fixative recommended by WHO [1992] for ejaculated sperm smears (ethanol:ether, 1:1). Spermatozoa from two other monkeys, released into PBS from region 1, were either smeared and air-dried, or fixed in 80% (v/v) ethanol, 80% (v/v) methanol or 5% (v/v) glutaraldehyde before smears were prepared. All smears were stained with Hemacolor™.

Computerized Assessment of Sperm Head Morphometry

The sperm image analysis software of the morphometric module of a Sperm-Class Analyzer® (SCA) computer-aided sperm analysis system (Microptic, Barcelona, Spain) was used. Hardware consisted of an Olympus BH-2 microscope equipped with a bright-field 100× oil immersion lens and a 3.3× photo-ocular lens; a CCD AVC-D7CE video camera (Sony Corporation, Tokyo, Japan); a 486 PC computer with an Intel 486 DX math coprocessor; a PIP-1024 B video digitizer board (Matrox Electronic Systems Ltd., Quebec, Canada) with a resolu-

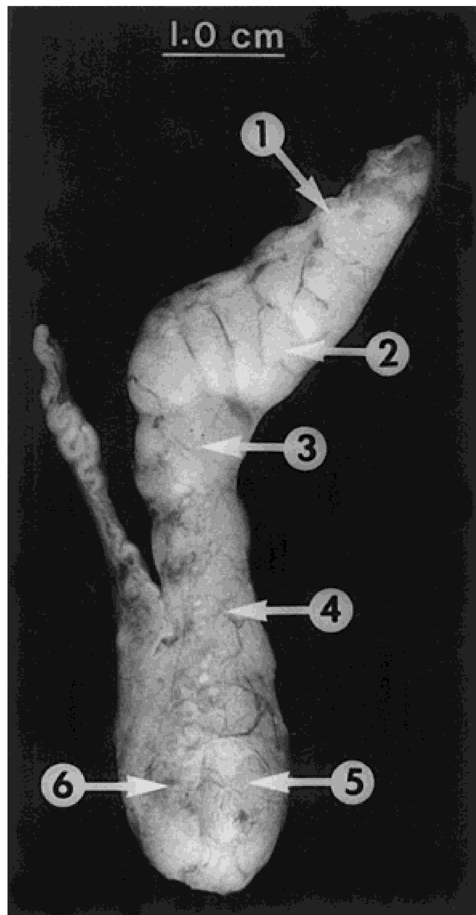

Fig. 1. Photomicrograph of the epididymis of a cynomolgus monkey, showing the regions from which spermatozoa were removed for morphological analysis. 1, initial segment; 2, caput; 3, proximal corpus; 4, mid-corpus; 5, proximal cauda; and 6, distal cauda. Magnification, see scale.

tion of 0.15 μm and 0.11 μm per pixel in the horizontal and vertical axes, respectively; and a high-resolution assistant monitor Sony Trinitron PVM-1443MD. The array size of the video frame grabber was 512 × 512 × 8 bits (picture elements, pixels). Final magnification on the videomonitor, after zooming, was 7000×. Before the observations were made, the illumination source was centered and a green filter (IF 550, Olympus) placed over the light source to enhance the contrast of the images. Intensity of the bulb and values of gain and offset of the camera were standardized across all samples.

Images of sperm heads were captured at random, with rejection only of those that overlapped. This process was performed manually by interactive selection of cells to avoid the inclusion of foreign particles that might interfere with subsequent processing of the image. Images of sperm heads were then digitized with a predetermined range of gray levels and sperm head boundaries were automatically defined and checked visually. Occasional incorrect definition of the sperm head boundary by the software was corrected manually with the aid of an editing facility. Measurements of size, shape, and mass (mean grey level of all pixels inside the boundary between 0 (black) and 225 (white)) were made automatically. Values were obtained for the following parameters area (μm^2, the sum of all pixel areas within the boundary), perimeter (μm, the sum of external bound-

aries), length (L) and width (W) (μm, the longest and shortest, respectively, of the feret diameters measured at angles of 0, 30, 60, 90, 120 and 150°, L and W not necessarily being orthogonal), and one shape factor (calculated from $4\ \pi A/P^2$, where A = area and P = perimeter) [Gago et al., 1999].

Classification of Morphological Forms

To evaluate the presence of artifacts, a cluster analysis was performed on cells from zone 1 of the epididymis of five control animals. The hierarchical method of clustering was used to separate different sub-populations of spermatozoa. Similarities between data measurements were considered as cosine distances and the furthest neighbor cluster method was employed. The best classification was obtained by considering three classes on which morphometric characterization was made. Using these data as reference values, a discriminant analysis was performed using the principal component analysis with the Mahalanobis method for the simulation of centroid distances. The classification matrix obtained was then applied to the matrix data of the whole sperm population (control and experimental, all epididymal zones) and percentages of each cellular type were calculated. For all calculations the Statistical Package for the Social Sciences™ (Jandel Scientific, Erkrath, Germany) was used.

Statistics

The normal changes occurring during epididymal maturation were obtained from the control males. Data on the percentages of different head forms were analysed for region-related changes by one-way ANOVA on ranks, followed by Dunn's test to determine which regions were different. The morphometric data were similarly compared for region-related differences by one-way ANOVA on raw data (perimeter) or after transformation (natural logs for area, reciprocals for length and width), followed by Tukey test.

The effect of antagonist treatment on morphometric parameters was examined by considering the two groups of males as belonging to three treatments: treatment for 16 days with vehicle (controls), and for 16 days and 25 days with Cetrorelix. Differences were assessed by performing two-way ANOVA considering both the treatment and the epididymal region. In the absence of region-treatment interaction, post hoc comparisons were made on all treatments (irrespective of region) and all regions (irrespective of treatment) by the multiple pairwise comparisons technique of Tukey. Differences were considered statistically significant when $P < 0.05$.

RESULTS

Morphological Examination of Maturing Epididymal Spermatozoa

When prepared for morphological staining in the manner considered routine for ejaculated human sperm cells, a variety of sperm head forms were observed (Figs. 2, 3). Sperm entering the epididymis had generally larger sperm heads with more intense staining than the majority in the cauda epididymidis. Two major sub-populations of abnormal sperm were classified as swollen (Fig. 3b,c,d) and highly swollen (Fig. 2a, Fig. 3e,f). In the first two proximal regions of the duct some extremely large sperm heads were observed (Fig. 2a, Fig. 3b) which gave the impression of overall swelling of a normal head shape (Fig. 3a). Other forms were recognizable as swelling from a predominantly post acrosomal re-

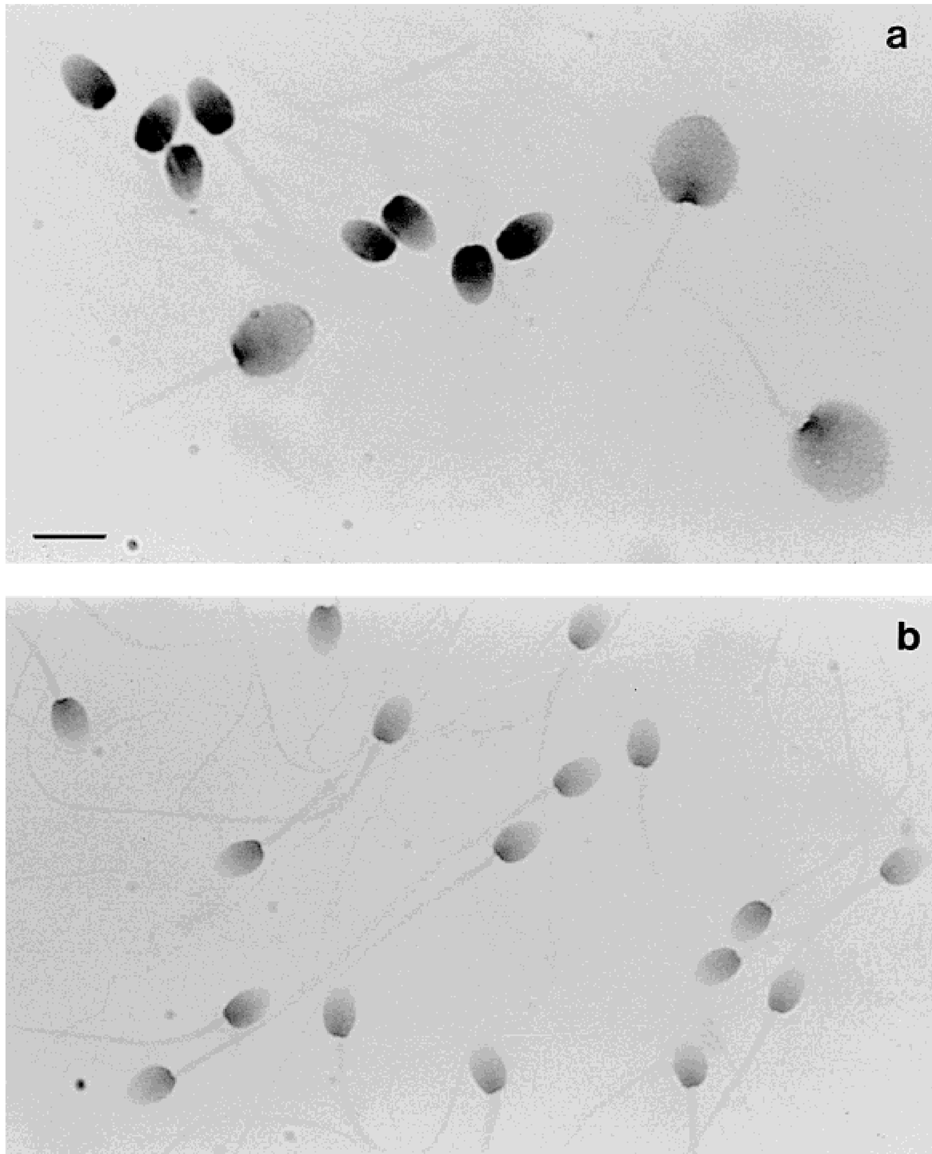

Fig. 2. Photomicrographs of spermatozoa from **a:** the caput and **b:** the cauda epididymidis of the monkey epididymis when air-dried as smears before fixing and staining. Note the larger sperm heads in the caput and some extremely swollen forms and the more normal appearance of sperm heads in the cauda. Bar in (a): 10 µm; also valid for (b).

gion, giving rise to acorn-shaped forms (Fig. 3c,d,e) which may develop into cloverleaf shapes (Fig. 3f).

Up to 15% of all spermatozoa were swollen in preparations from the first three epididymal regions under the usual conditions of cell preparation, i.e. when the cells were immediately released into PBS, smeared and air-dried before fixation and staining. However, these giant forms were not seen in region 2, but were still present in region 1 when ethanol was used before the smears were

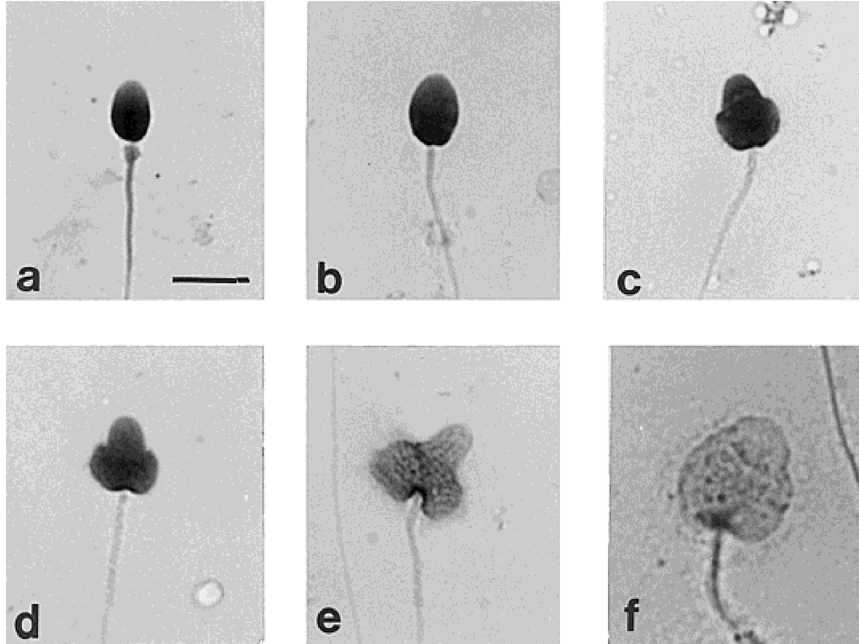

Fig. 3. Montage of video images of sperm heads from proximal regions of the epididymis of control monkeys, displaying the known varieties of swollen and distended forms. **a:** normal, **b:** swollen, **c, d:** swollen, acorn-shaped, **e, f:** highly swollen. Bar in (a): 10 μm; also valid for (b–f).

prepared. Thus, in one monkey examined in detail, the percentages of morphologically abnormal sperm heads, including swollen and highly swollen forms from regions 1, 2, 3, 4, 5, and 6 of the epididymis were, respectively, 15, 7, 3, 0, 0, and 0 when unfixed, and 20, 0, 0, 0, 0, and 0 when fixed in ethanol before air drying (300 cells were assessed per zone and fixative).

Assessment of spermatozoa was difficult because treatment with ethanol caused agglutination of spermatozoa at the edge of the slides. Interpretation of results was also unclear because ethanol could dehydrate sperm and reduce their size before fixation. Thus, fixation in methanol and glutaraldehyde was used for further analysis of sperm from zone 1, where the highest proportion of such swollen forms in unfixed preparations was observed. The percentages of morphologically abnormally large sperm heads (slightly swollen and highly swollen forms) found in region 1 of the epididymides of two monkeys were 42% and 35% when unfixed, and 45% and 0% or 30–40% and 7% when fixed in methanol or ethanol, respectively. Both methanol and ethanol induced agglutination of sperm cells, but some swollen forms were still visible. In contrast, fixation with glutaraldehyde produced no agglutination in either monkey, and it completely abolished the appearance of all swollen cells.

Morphological Characterisation of the Swollen Forms

Morphometric analysis of the sperm heads was made with respect to area, perimeter, length, width, and shape factor of cells from zone 1 of five control animals. This database was examined by cluster analysis, which generated three classes termed normal, swollen and highly swollen, (Table 1). Both types of swol-

TABLE I. Morphometric Characteristics of the Three Populations of Spermatozoa Obtained After Cluster Analysis of Sperm Heads From Epididymal Region 1

	Normal (n = 157)	Swollen (n = 269)	Highly swollen (n = 59)
Area (μm^2)	17.48 ± 2.64	27.50 ± 5.04	48.36 ± 4.99
Perimeter (μm)	15.52 ± 1.08	19.35 ± 1.95	26.23 ± 1.57
Length (μm)	5.81 ± 0.44	7.13 ± 0.61	9.21 ± 0.51
Width (μm)	4.10 ± 0.35	5.26 ± 0.62	7.34 ± 0.51
Shape	1.34 ± 0.90	2.15 ± 1.35	4.90 ± 2.30

Values are means ± SD.

len cells were 1.5 to 3 times the area of the normal sperm heads, with associated greater perimeter, length, and width. Shape factor was the greatest discriminator of some 3.6-fold.

Morphological Changes in the Sperm Head During Epididymal Passage

Using the classification matrix obtained from the cluster and discriminant analysis, it was possible to ascertain the percentage of each of these forms that were produced after morphological preparation of sperm taken from each epididymal region. These percentages are presented in Figure 4, in which it can be seen that on the control males there were more swollen forms than highly swollen forms, but only in epididymal regions 1 and 2, where non-swollen forms accounted for about 30% of the cells. The low percentage of highly swollen cells in region 1 had nearly vanished by region 3, and swollen cells decreased from 68% to 13% between regions 2 and 3. As a corollary, the percentage of sperm with normally-shaped heads increased sharply from 29% to 87% between regions 2 and 3. Kruskall-Wallis one-way ANOVA on ranks indicated that there were significant differences (H = 22.3, df = 5) in the percentages of swollen forms between epididymal regions 1/2 and regions 5/6, as was also found for the non-swollen forms (H = 22.9, df = 5), but no regional differences in the highly swollen forms were detected (H = 19.8, df = 5).

Following treatment with GnRH antagonist for 16 days, the greatest percentages of highly swollen cells were also present in epididymal regions 1 and 2, but they were greatly variable between animals. As was found for the controls, these forms had largely disappeared by region 3. Swollen forms declined sharply from 58% to 1% between regions 3 and 4, more distally than the sharp decline in swollen cells for controls.Consequently, the prominent increase in normal forms after 16 days of antagonist treatment from 41% to 99% was also delayed in comparison to controls. Following treatment with GnRH antagonist for 25 days, the low percentages of highly swollen forms found in regions 1 and 2 essentially vanished by region 3; swollen forms, however, decreased slowly across regions following 25-day antagonist treatment, in parallel with the increase in normal forms.

Changes in Non-Swollen Forms Upon Epididymal Transit

It was readily apparent that spermatozoa not characterised as being swollen from the cluster analysis and whose morphology was uniform, suffered slight but significant changes as they passed through the epididymis, and that antagonist treatment prevented some of these changes from occurring (Fig. 5). One-way analysis of variance performed on the data from control animals revealed significant region-dependent differences for sperm head length (F = 7.97, df = 5), head width

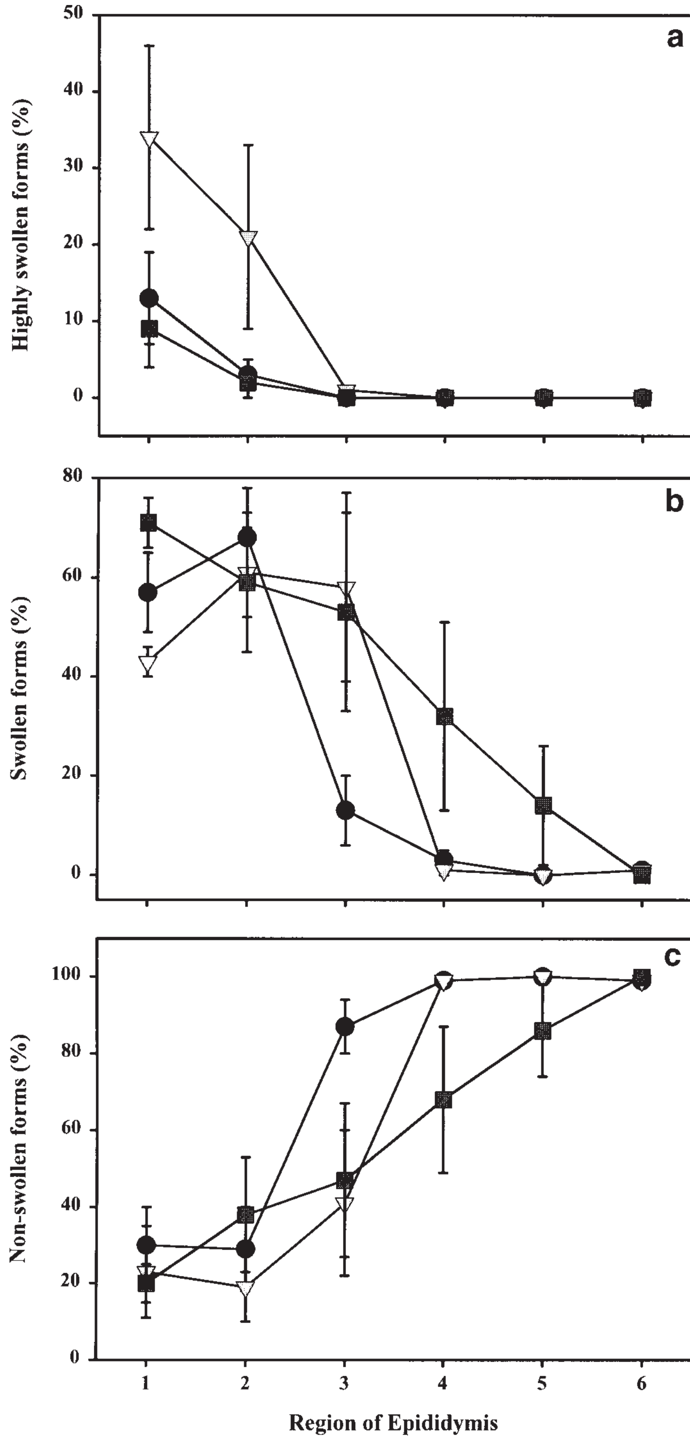

Fig. 4. Percentages (ordinate, mean ± sem) of spermatozoa with **a:** highly swollen, **b:** swollen and **c:** non-swollen heads from different regions of the epididymis (abscissa) of control monkeys (●) or the GnRH-antagonist Cetrorelix for 16 days (▼) or 25 days (■).

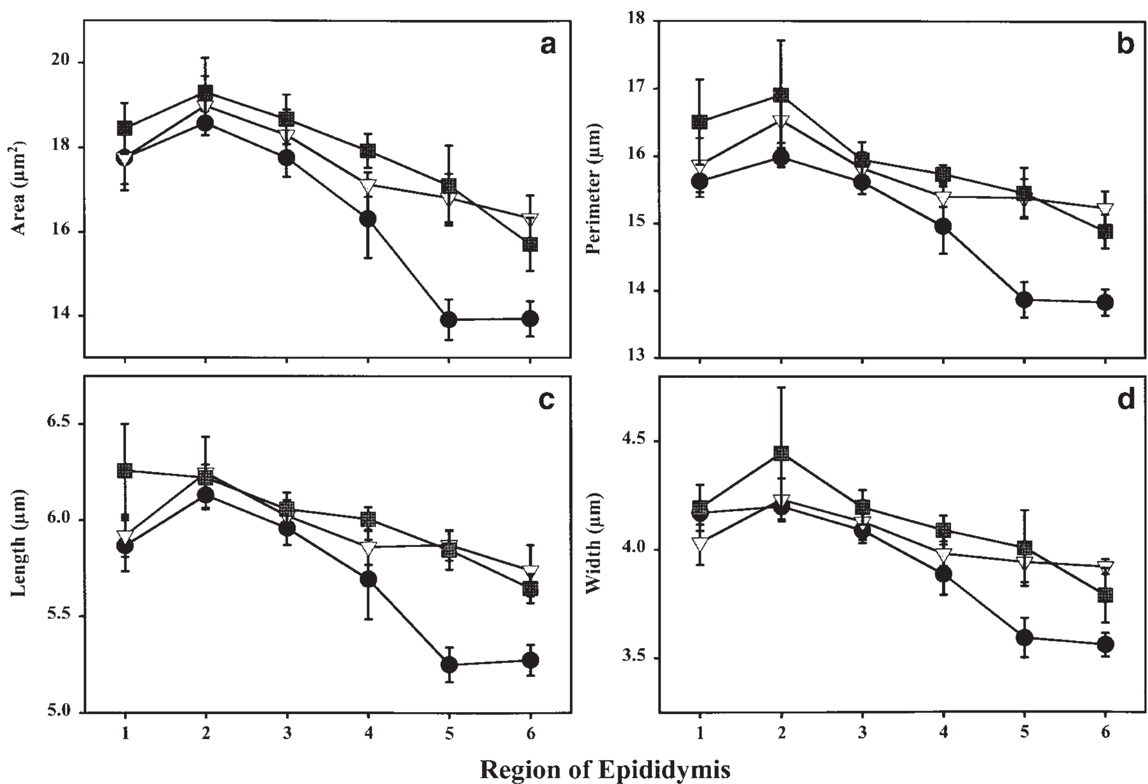

Fig. 5. Morphometric parameters of sperm heads (ordinate, mean ± sem) [**a:** area (μm^2), **b:** perimeter (μm), **c:** length (μm), **d:** width (μm)] for sperm from different regions of the epididymis [(1) initial segment, (2) the caput region where the tubule was more darkly pigmented than adjacent regions, (3) proximal corpus, (4) mid-corpus, (5) proximal cauda, (6) distal cauda epididymidis] from five control animals (control ●) or GnRH-antagonist Cetrorelix for 16 (▼) or 25 (■) days.

(F = 17.41, df = 5), perimeter (F = 15.53, df = 5), and area (F = 14.30, df = 5). The values for all these parameters for sperm from epididymal regions 1, 2, and 3 were significantly greater than those for sperm obtained from regions 5 and 6. This describes the normal maturational process of sperm head shrinkage by which morphologically normal sperm attain their most mature state in region 5 (the proximal cauda epididymidis), which persists in the distal cauda epididymidis (region 6).

The size of sperm heads from the males having received Cetrorelix for 16 or 25 days also declined as they traversed the epididymis, but the decline was not so steep as in the controls. Two-way analysis of variance of all the data from controls and Cetrorelix-treated males revealed significant differences between regions (df = 5) for sperm head area (F = 11.15), length (F = 9.46), width (F = 9.37), and perimeter (F = 10.51). Independent of the treatment, the values in epididymal regions 1, 2, and 3 were significantly greater than those for sperm from regions 5 and 6. ANOVA also revealed significant treatment-related differences (df = 2) for sperm head area (F = 5.89), length (F = 7.40), and perimeter (F = 8.36). Independent of the region, there were significant differences between control and Cetrorelix treatment at both time points. There was no treatment-related effect on sperm head width (F = 3.42), and no interaction between region and treatment for any parameter.

DISCUSSION

This is the first study to use an image analysis system to assess the morphology of maturing monkey epididymal spermatozoa. The characteristics of mature cauda epididymidal spermatozoa reported here resemble those of the ejaculate previously published [Gago et al., 1999], prepared under the same conditions. Although these conditions included air-drying before fixing and staining, which damages the plasma membranes of human spermatozoa [Haas et al., 1988], the results suggest that mature epididymal spermatozoa are not grossly influenced by the addition of accessory gland secretions at ejaculation in response to the processes of air-drying, fixing, and staining.

In contrast, immature spermatozoa were shown to be extremely sensitive to the air-drying process, and a minority was deformed when they were subsequently examined after fixing and staining. The large values for perimeter and area of sperm heads obtained from the proximal epididymis resemble sperm from the proximal human and monkey epididymis that have been described as ghosts or swollen forms by Yeung et al. [1997]. These were also prepared by making air-dried sperm smears before subsequent staining, but because human epididymal sperm were not plentiful, experiments on the cause of the swelling were performed on cynomolgus monkey sperm as a related non-human primate species. It was demonstrated that ethanol fixation prior to preparation of smears and air-drying of monkey sperm prevented the appearance of such forms. Although ethanol was used because it is the major fixative used on air-dried smears in the WHO technique, it could reduce cell size as a consequence of cellular dehydration; therefore, in this study other fixatives were compared to ethanol. While glutaraldehyde was completely effective, we were unable to confirm the abolition of all types of swollen forms with ethanol or methanol. The results confirm that the abnormally large heads of epididymal spermatozoa are a consequence of the preparation technique and should not be considered the state of the cells within that region of the epididymis in situ.

It is interesting that in our previous work on epididymal sperm from other

species: [rabbit: Pérez-Sánchez et al., 1997, 1998; mouse: Tablado 1996; guinea pig: Cooper et al., 2000] this type of swollen immature cell was hardly ever observed, even though the samples were prepared in the same way as here, namely, by air drying before fixation. In contrast, they are observed in both man and cynomolgus monkey [Yeung et al., 1997, 1999], raising the possibility that this could be a peculiarity of immature primate cells that have a propensity to distortion.

The reason for the swelling of unfixed immature primate sperm is not known. It is not observed in unfixed wet preparations (during video-recording for motility estimation (unpublished observations)), suggesting that it occurs during air-drying. However, evaporation of the medium during this process would lead to extra-cellular salt deposition and these hypertonic conditions should draw water out of the cells leading to shrinkage rather than swelling. Observation of unstained cells after drying is difficult because of the presence of salt deposits. It is possible that during the subsequent fixation and staining in aqueous medium, extracellular salts are washed away and water may then be drawn osmotically into the now hypertonic cells, leading to the observed cell swelling. Since the post-acrosomal region is affected more often than the acrosomal area, either the acrosomal cap is protective, preventing expansion of the acrosomal region, or the post-acrosomal region is intrinsically less stable. It is also possible that sperm membranes damaged by any initial shrinkage are particularly susceptible to subsequent rehydration. Whatever the cause, it is clear that future morphological studies on primate epididymal spermatozoa should involve fixation of cells before smears are made in order to avoid generating unnatural forms not present in the epididymal lumen. However, the sensitivity to distortion upon air-drying by the immature cells does provide a method of detecting cells that have not undergone epididymal maturation.

An earlier subjective analysis of the maturational profile of normal, non-swollen monkey epididymal sperm indicated that they became the sole cell type in epididymal region 3 [Yeung et al., 1999]. In this study, the maturation of sperm cells that had resisted artifactual distortion was studied objectively by analysing only the non-swollen cells. All the morphometric parameters measured for control males indicated a general decrease in head size upon maturation, with their minimum size being attained in the proximal cauda epididymidis. Decreases in monkey sperm size during epididymal maturation has been documented before, using far fewer cells, by standard morphometric and electron microscopic techniques [Bedford & Nicander, 1971; Moore et al., 1984]. The decrease in sperm head size occurs in more proximal epididymal regions in the rabbit [Pérez-Sánchez et al., 1998].

This reduction in sperm head size could be secondary to the post-testicular condensation of the sperm chromatin, which is also shifted to more distal monkey epididymal regions after gonadotrophin withdrawal [Golan et al., 1997]. This suggestion is supported by the decrease in sperm nuclear size (length, width, area) that occurs between the human caput (immature sperm) and cauda epididymidis (mature sperm) in parallel with an increase in nuclear condensation [Auger & Dadoune, 1993]. On the other hand, studies on ejaculated human spermatozoa indicate that head shape anomalies of these mature sperm are not caused by factors that influence the volume of sperm chromatin (DNA organization or compaction) since the nuclear volume remains the same [Lee et al., 1997]. Although physical damage to human spermatozoa can lead to spatial disarrangement of chromatin that resembles the swelling seen upon decondensation [Zuccotti et al., 1994], the anomalous sperm heads seen in the human ejaculate [Lipitz et al.,

1992; Francavilla et al., 1996] are neither the leaf-like nor the acorn-like forms observed in some immature epididymal sperm [Yeung et al., 1997, this work].

Cell shrinkage could possibly also reflect a marginal cellular dehydration as a consequence of the cells entering a hypertonic epididymal environment [Cooper, 1986], but the osmolality of monkey epididymal fluid has not been measured. If dehydration is the driving force for the cell compaction observed during maturation of sperm in normal epididymides, then the present work may indicate that the composition of luminal fluid is altered by GnRH-antagonist treatment.

The effects of GnRH-antagonist treatment on subjective evaluation of sperm morphology indicated that the ascendance of normal forms occurred in the same epididymal region despite a lowered epididymal androgen level [Yeung et al., 1999]. In this report, a different categorization of normal forms indicated a more distal location of normal forms in the antagonist-treated males as determined before [Yeung et al., 1999], and the lack of swollen forms in the cauda epididymidis (region 6) was confirmed. This indicates that the major observable changes on sperm morphology can occur in the presence of lower than normal amounts of androgens.

The greater power of computerized methods revealed for the first time that significant changes occurred to the head shape of normal spermatozoa during their transport through the epididymis in untreated males. Furthermore, the reduction in androgens by Cetrorelix treatment led to a less than normal decline in head size upon the maturation, reflecting the androgen-dependent nature of this maturational process, with the result that the sperm in the cauda epididymidis, destined for ejaculation, were larger than normal. Abnormalities in the morphology of ejaculated spermatozoa from the cynomolgus monkey may reflect androgen-dependent damage occurring in the epididymis or epididymal effects of environmental pollutants that may also have an effect on the human species.

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