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Abnormal morphology of vervet monkey sperm

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Abstract: Ejaculates of 14 colony-bred and 14 wild-caught vervet monkeys were examined for morphologically abnormal sperm. Sperm head abnormalities were rare in both groups, occurring at rates of 0.01–0.29%. Tail abnormalities predominated, particularly bent midpieces and coiled and folded tails, which all occurred at rates of 3.79–17.18%. Except for the nipple acrosome, there was no difference in the rate at which sperm abnormalities were found in both groups. Three abnormalities were found only in colony-bred and three only in wild-caught individuals.

Some common abnormalities, all affecting the tail, were highly variable in consecutive ejaculates from the same individuals.

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Introduction

The description of abnormal morphology is an essential part of defining the sperm characteristics of any mammal. Not only because different abnormalities may predominate in different species, but because baseline data will also assist in the diagnosis of pathology of the reproductive system and are useful in biomedical research.

An investigation should include establishing the types of abnormal forms, the rate at which they occur, and their prevalence. Such data have been well established for human sperm and that of a number of domestic animal species [10,18,21]. On the other hand, many studies on the semen characteristics of nonhuman primates appear to be confined to investigating sperm concentrations and motility and at times total normal or abnormal morphology [1,3,4,9,11–14,17,23–25,28]. Specific defects may sometimes be mentioned but not quantified [4,25]. A lack of data is particularly evident for nonhuman primates which are not commonly maintained in the laboratory, such as the vervet monkey (*Cercopithecus aethiops*). This article provides a detailed description of abnormal sperm morphology of vervet monkeys of captive-bred and wild-caught origin.

Materials and methods

An ejaculate was collected from each of 14 captive-bred and 14 wild-caught vervet monkeys by electrostimulation, as described previously [25]. The wild-caught individuals had been maintained in the colony for at least five years prior to sampling. The criteria for inclusion were sexual maturity and the absence of overt pathology of the reproductive system and genitals.

Two additional ejaculates were taken separately at two-week intervals from ten males, regardless of their origin. These serial samples were obtained to examine intra-individual variability of abnormal morphology.

Housing, environmental conditions, and nutrition

All individuals were housed singly and permanently indoors in 0.6 × 0.6 × 0.8m and 1.2 × 0.6 × 0.8m wall-mounted stainless steel home cages. Access to 0.6 × 0.6 × 2.0m exercise cages was provided every three to six days for 24 hours. Each exercise cage also contained a female social partner. The home cages were fitted with perches, foraging containers, and sterilized cattle femurs for manipulation. Details of air conditioning, photoperiod, supply of drinking water, and diet have been described previ-

ously [26]. The diet has supported good reproductive performance through two generations.

Preparation and evaluation of sperm smears

It was anticipated that coiled and bent tails and midpieces would be easily visible microscopically in fresh semen. Therefore, the presence of these abnormalities was determined qualitatively in a wet preparation under bright field illumination at 400× magnification. Abnormalities in the wet preparations were rated as absent or present in small, moderate, and large numbers. This was later compared with the results obtained from stained specimens to ascertain that these abnormalities were not artifacts of processing, since tail coiling can be caused by drying, cooling, or contamination of the sample and hypo-osmotic shock [11,30].

For the quantitative determination of all defective forms, a thin semen smear was made on a glass slide and air dried for approximately five min. This was followed by staining with Spermac stain (Stain Enterprises, Wellington 7655, Republic of South Africa) according to the manufacturer's instructions. The staining procedure included the following steps:

1. Immersion of the smear in a formaldehyde-based fixative for 30 min.
2. Rinsing slides by gently dipping in tap water.
3. Staining for 1 min in stain A and rinsing in tap water.
4. Staining for 45 sec in stain B and rinsing in tap water.
5. Staining for 1 min in stain C and rinsing in tap water.

Spermac stain has been tested in a number of species, including dogs, vervet monkeys, and humans and allowed differentiation of the acrosome, the post-acrosomal region and tail [5,20–22]. The post-acrosomal region and nuclear portion of the head stains red, and the acrosome midpiece and tail, green.

Table 1. Abnormal forms of sperm from vervet monkeys

Head:	macrocephalic (c+w), microcephalic (c+w), round no acrosome (c), narrow (c+w), acrosomal cysts (c+W), equatorial cysts (c+w), nipple acrosome or apical aggregation of acrosomal material (c+w), pointed (c+w), asymmetrical (c), duplication (c+w), pyriform (c+w), tapered (c+w), amorphous (c).
Midpiece:	bent (c+w), thickened (c+w), abaxial implantations (c+w), cytoplasmic droplets (c+w), bent neck (w), pseudodroplet defect or mitochondrial stripping (w), duplication (w).
Principal and endpiece:	Coiled (c+w), folded (c+w), detached at midpiece (c+w), detached endpiece (c+w), duplication (c+w), terminal coiling (c+w), detached (c+w), bent (c+w).

Key to letters in parentheses: c = occurred in colony-bred individuals, w = occurred in wild-caught individuals.

For the determination of the abnormal morphology, 200 spermatozoa were rated from each smear, according to the following categories.

Head abnormalities

Any deviations from the normal oval shape, size, and duplications were recorded; particularly, but not restricted to: micro- and macrocephalic, asymmetrical, round, elongated, narrow, amorphous, tapering, pyriform, and detached heads, vacuoles, cysts and invaginations [30]. Where possible, the defects were evaluated within the separate regions of the head, including the acrosome, equatorial segment, and post-acrosomal region.

Tail abnormalities

Defects were, where possible, recorded within the distinct regions of the tail and included the following categories [30]:

Table 2. Head abnormalities found in 14 ejaculates from 14 colony-bred vervet monkeys

Defect	No. of males with defect (%)	Mean rate* (%)	Mean rate** (%)
Macrocephalic	4 (28.6)	0.21 ± 0.38	0.75
Microcephalic	3 (21.4)	0.11 ± 0.21	0.50
Round, no acrosome	2 (13.3)	0.07 ± 0.18	0.50
Narrow	4 (28.6)	0.18 ± 0.32	0.63
Acrosomal cysts	2 (14.3)	0.11 ± 0.29	0.75
Equatorial cysts	1 (7.1)	0.04 ± 0.13	0.50
Nipple defect	7 (50.0)	0.29 ± 0.32	0.57
Pointed	2 (14.3)	0.07 ± 0.18	0.50
Asymmetrical	3 (21.4)	0.29 ± 0.80	1.33
Duplication	3 (21.4)	0.11 ± 0.21	0.50
Pyriform	1 (7.1)	0.04 ± 0.13	0.50
Tapered	2 (14.3)	0.07 ± 0.18	0.50
Amorphous	2 (14.3)	0.07 ± 0.18	0.50
Total		1.64 ± 1.39	

*Generated from entire group.
**Generated from males with specific defect.

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Table 3. Head abnormalities found in 14 ejaculates from 14 wild-caught vervet monkeys

Defect	No. of males with defect (%)	Mean rate* (%)	Mean rate** (%)
Macrocephalic	1 (7.1)	0.01 ± 0.05	0.20
Microcephalic	4 (28.6)	0.21 ± 0.37	0.73
Narrow	4 (28.6)	0.21 ± 0.38	0.75
Acrosomal cysts	4 (28.6)	0.21 ± 0.38	0.75
Equatorial cysts	2 (14.3)	0.11 ± 0.29	0.75
Nipple defect	2 (14.3)	0.07 ± 0.18	0.50
Pointed	1 (7.1)	0.07 ± 0.27	0.75
Duplicate	2 (14.3)	0.11 ± 0.29	0.75
Pyriform	2 (14.3)	0.07 ± 0.18	0.50
Tapered	2 (14.3)	0.07 ± 0.18	0.50
Total		1.15 ± 1.02	

*Generated from entire group.
**Generated from males with specific defect.

Neck. Abaxial implantations, broken, detached heads.
Midpiece. Bends, kinks, thickening, mitochondrial stripping or displacement, detachment, duplication, vacuoles, and cysts.
Principal- and endpiece. Coiling, folding, bends, kinks, thickening, detachment, and duplication.

All results were expressed as percentage of counted spermatozoa. Morphologically abnormal forms were photographed with an Olympus BH 2 microscope with automatic micrographic system model PM-10 ADS and a PM-CTR colour temperature module. The objective was a PLAN Apochromatic 100-1.3 oil, the eyepiece of a 3.3 NFK photo, and the film used a 50 ASA FUJI daylight colour reversal film. A blue filter was placed over the light source and the exposure was 1 sec.

Statistics

The difference of specific types of abnormal sperm between colony-bred and wild-caught individuals was determined by Wilcoxon's 2-sample test.

Results

A total of 28 different sperm abnormalities were found among the ejaculates of 28 vervet monkeys. From a total of 13 head abnormalities, three types were found only in colony-bred individuals. From a total of seven midpiece abnormalities, three types were found only in wild-caught individuals, but all eight types of principal- and endpiece abnormalities occurred in both groups. Table 1 lists all defective forms found per morphological area and according to the vervet monkey's origin.

The qualitative evaluation of the wet preparations could be related to the quantitative evaluation of the stained smears.

All head abnormalities of colony-bred and wild-caught males are summarized in Tables 2 and 3, respectively. It can be seen that none occurred at a rate of more than 1% in both groups, with one exception: the asymmetrical head. A total of 13 types of head abnormalities were found in colony-bred individuals and ten in wild-caught ones. In the former, the nipple defect was the most prevalent, occurring in 50% males, whereas in the latter it was the narrow head, the acrosomal cyst, and the microcephalus, each found in 28.6% males. All three of these defects also occurred at the highest mean rates.

Four and seven midpiece abnormalities were identified in colony-bred males and wild-caught males, respectively. The most prevalent were bent

Table 4. Midpiece abnormalities found in 14 ejaculates from 14 colony-bred vervet monkeys

Defect	No. of males with defect (%)	Mean rate* (%)	Mean rate** (%)
Bent	9 (64.3)	5.75 ± 6.71	8.94
Thickened	7 (50.0)	0.68 ± 1.32	1.44
Abaxial implantations	5 (35.7)	1.54 ± 2.89	4.50
Cytoplasmic droplets	2 (14.3)	0.04 ± 0.13	0.50
Total		8.00 ± 7.29	

*Generated from entire group.
**Generated from males with specific defect.

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Table 5. Midpiece abnormalities found in 14 ejaculates from 14 wild-caught bred vervet monkeys

Defect	No. of males with defect (%)	Mean rate* (%)	Mean rate** (%)
Bent	10 (71.4)	8.57 ± 10.55	12.00
Head bent at neck	2 (14.3)	0.07 ± 0.18	0.50
Thickened	8 (57.1)	0.82 ± 1.23	1.44
Abaxial implantations	7 (50.0)	0.32 ± 0.37	0.64
Pseudodroplet defect	1 (7.1)	0.07 ± 0.27	1.00
Cytoplasmic droplet	2 (14.3)	0.11 ± 0.29	0.75
Duplicate	1 (7.1)	0.04 ± 0.13	0.50
Total		10.00 ± 11.11	

*Generated from entire group.
**Generated from males with specific defect.

midpieces, which occurred in 64.3% of the colony-bred males and 71.4% in wild-caught males. This defect also occurred at the highest mean rate in both groups. The rate and prevalence of all other abnormalities was considerably lower, and all results are summarized in Tables 4 and 5. Abaxial implantations and thickened midpieces were also common in both groups; however, these and the other defects occurred at a rate of less than 2%.

Out of a total of eight tail abnormalities found in colony-bred and wild-caught males, coiled and folded tails were the most prevalent in both groups. In colony-bred males they occurred in 92.9% and 100% of males, respectively, and in wild-caught 85.7% and 92.9%, males, respectively. Coiled tails also occurred at the highest mean rate in both groups. Tables 6 and 7 summarize all results.

Summary of tables

Figures 1–2 summarize the results from Tables 2–7. Principal-piece abnormalities were found to occur at the highest mean rate among both wild-caught and colony-bred monkeys. The rate at which midpiece abnormalities occurred in both groups was considerably lower than principal-piece defects. Head abnormalities occurred at the lowest rate in both groups. Most types of defects were found in the head region. This was followed by the principal- and midpiece region, in that order. Large standard deviations from the means of all defects among all males reflect the large individual differences.

Serial morphology

The results of the morphological evaluation of ten ejaculates taken twice from ten males at an interval of two weeks are summarized in Tables 8 and 9. Considerable differences could be observed between two ejaculates of most individuals.

In 60% of males, differences of over 100% were recorded in consecutive ejaculates for coiled tails (Table 8). Folded tails differed by more than 100% in 50% of males (Table 8).

Detached tails differed by more than 100% in consecutive ejaculates of 90% of males (Table 9) and bent midpieces by more than 100% in consecutive ejaculates of 70% of males (Table 9).

Out of four males with the consistently highest number of coiled tails (20–66%), two are successful breeders. The male with the highest number of folded tails is a successful breeder. Out of two males with the highest number of detached tails (17–45%),

Table 6. Tail abnormalities found in 14 ejaculates from 14 colony-bred vervet monkeys

Defect	No. of males with defect (%)	Mean rate* (%)	Mean rate** (%)
Coiled	13 (92.9)	17.18 ± 19.98	18.50
Folded	14 (100.0)	7.00 ± 9.02	7.00
Detached at midpiece	2 (14.3)	0.07 ± 0.18	0.50
Detached at endpiece	2 (14.3)	0.14 ± 0.41	1.00
Duplication	1 (7.1)	0.07 ± 0.27	1.00
Terminal coiling	5 (35.7)	0.68 ± 1.62	1.90
Bent	1 (7.1)	0.29 ± 1.07	4.00
Detached	14 (100.0)	3.89 ± 3.91	3.89
Total		29.32 ± 27.42	

*Generated from entire group.
**Generated from males with specific defect.

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Table 7. Tail abnormalities found in 14 ejaculates from 14 wild-caught vervet monkeys

Defect	No. of males with defect (%)	Mean rate* (%)	Mean rate** (%)
Coiled	12 (85.7)	16.54 ± 15.33	21.63
Folded	13 (92.9)	3.79 ± 2.12	4.62
Detached at midpiece	4 (28.6)	0.71 ± 1.55	2.50
Detached at endpiece	2 (14.3)	0.21 ± 0.58	1.50
Duplication	4 (28.6)	0.29 ± 0.58	1.00
Terminal coiling	5 (35.7)	0.79 ± 1.41	1.92
Bent	1 (7.1)	0.04 ± 0.13	0.50
Detached	11 (78.6)	7.00 ± 5.72	7.32
Total		29.36 ± 17.20	

*Generated from entire group.
**Generated from males with specific defect.

one male is a successful breeder, while both males with the highest number of bent midpieces (Table 9) are successful breeders.

Difference between wild-caught and colony-bred males

The only parameter for which a statistically significant difference existed for both groups was the nipple acrosome ($p < 0.05$). No statistical difference could be demonstrated for any of the other abnormalities which occurred in both groups.

Discussion

The prevalence and rate of specific morphological abnormalities of sperm from wild-caught and colony-bred vervet monkeys is provided for the first time. Most features, including the rate of abnormal morphology, the types of abnormal forms, and the prevalence of tail abnormalities agree with what has been reported for other Old World and New World nonhuman primates [4,6,11,12,19,27]. Head defects appeared to be rare in all taxa investigated, which agrees with what has been found in this study for vervet monkey sperm. Here, the most common head abnormalities were the nipple acrosome and microcephalus in colony-bred and the microcephalus, narrow head, and acrosomal cysts in wild-caught individuals. All occurred however at a rate of < 1%. A low rate of head abnormalities has also been reported for the sperm of marmosets, tamarins, and crested macaques [6,12,27]. Only three types of head abnormalities were observed in marmosets, including macro- and microcephalic and amorphous forms which included narrow, tapering, and round.

Head abnormalities are caused during spermatogenesis and spermiogenesis [15,16]. The low rate of head abnormalities in nonhuman primates could therefore imply that these processes fail more often in humans.

Among all types of abnormalities, coiled, folded, and detached tails occurred at the highest rate and prevalence in vervet monkeys. The high rate of coiled and folded tails, as well as bent midpieces in the vervet, could be confirmed qualitatively in fresh unstained specimens. Tail abnormalities can be

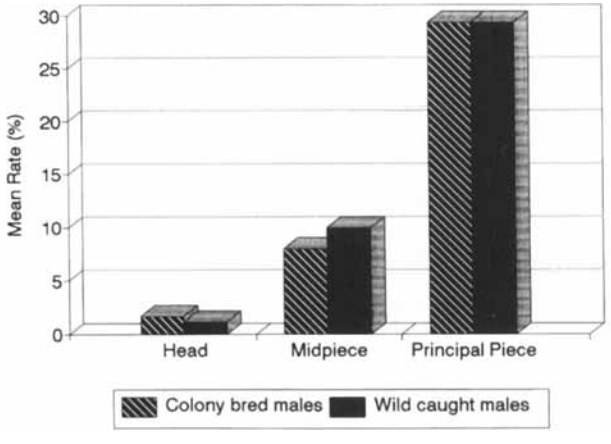

Fig. 1. Average rate of defects per morphological structure.

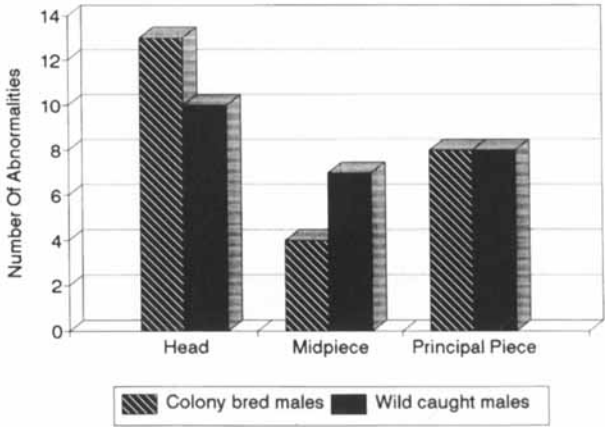

Fig. 2. Number of difference defects found.

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Table 8. Coiled and folded tails in ten ejaculates from ten males taken twice at an interval of two weeks

	Coiled tails (%)		Folded tails (%)	
	Ejaculate		Ejaculate	
Number	1	2	1	2
46	52	20	5	5
879	1	4	2	3
889	10	19	0	5
660	0	18	0	29
940	5	45	17	3
934	43	35	8	3
777	66	63	6	8
563	28	10	5	4
884	18	33	4	8
754	50	20	12	11
Mean	27.30	26.65	5.90	7.90
± SD	24.01	17.65	5.32	7.88

artifacts of drying, cooling, and contamination or the results of hypo-osmotic stress [11,30]. Coiled and kinked tails were also common in sperm from Sulawesi macaques and Capuchins [4,27]. On the other hand, the most frequent abnormalities in sperm of tamarins were midpiece defects. Coiled tails, cytoplasmic droplets, and detached heads were also observed, but in very small numbers.

Abnormal spermatozoa were relatively rare in cynomolgus monkeys and less than 10% of all ejaculates contained more than 30% abnormal forms, including 15% bent and 8% kinked tails [19]. Only tail abnormalities appear to have been observed during this study.

The different types of morphologically abnormal forms found in vervet monkey sperm were of the same types described previously for this and other nonhuman primate species [6,11,12,19,25].

Table 9. Detached tails and bent midpieces in ten ejaculates from ten males taken twice at an interval of two weeks

	Detached tails (%)		Bent midpieces (%)	
	Ejaculate		Ejaculate	
Number	1	2	1	2
46	10	0	6	3
879	22	8	6	9
889	32	17	0	8
660	45	1	2	14
940	8	10	0	15
934	3	7	2	16
777	1	13	1	12
563	6	14	20	13
884	0	1	35	13
754	14	1	3	18
Mean	14.10	7.10	7.45	13.40
± SD	14.72	6.36	11.35	6.22

A low rate of head abnormalities in nonhuman primate sperm is agreed upon by all authors, whereas the data on gross abnormal morphology are conflicting. Some found nonhuman primate sperm to be relatively free from morphological abnormalities, with a range of 0–20% defective forms [4,11,12,25,29]. This includes data from a study carried out in this facility. Others report higher rates for many species of up to and over 50% abnormal [1,3,6]. This is likely to be due to species-specific differences in some cases and a lack of standardized methods and systems in others. But there are also conflicting reports involving the same species, such as in the capuchin [1,4].

Other authors have reported that abnormal forms found among vervet sperm range from 24% to 40% and 0% to 32% [1,28,29]. In this study, the total abnormal morphology of captive-bred individuals was 38.96%, and wild-caught 40.51%, thus supporting the higher range of abnormal sperm (Tables 2–7). But tail abnormalities, particularly coiled and folded tails, contribute over 70% to this rate, which, as previously mentioned, may be artifacts of processing. This could, therefore, artificially inflate the figure for total abnormal morphology.

Only four of the most common abnormalities, all affecting the tail, were investigated in the consecutive ejaculates. The rates at which head defects occurred were too low to produce meaningful comparisons and results. All four abnormalities, which included coiled tails, folded tails, detached tails, and bent midpieces, were highly variable within all individuals. Considerable variations in the gross normal morphology of two ejaculates from the same individuals have also been reported by other workers in vervet monkeys [29]. The results demonstrate that a single or even two ejaculates cannot be representative of an individual's reproductive potential.

Since most individuals with the highest abnormal morphology were highly successful breeders, abnormal morphology appears to have had no effect on fertilization in the vervet monkey. However, only tail abnormalities occurred in large numbers.

Except for one defect, there was no difference between captive-bred and wild-caught individuals. Although this was not expected, differences might nevertheless have been produced by different stress levels, nutrition, and exposure. The nipple defect was the only abnormality found in a larger number of colony-bred males, and at a considerably higher rate, than in wild-caught individuals. This abnormal form consists of an apical aggregation of acrosomal material, sometimes forming a cyst. A fault during the cap formation phase of spermiogenesis might be the reason for such defects.

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