Onderstepoort Journal of Veterinary Research, 61:239–246 (1994)

Ultrastructure of ostrich *(Struthio camelus)* spermatozoa. II. Scanning electron microscopy

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ABSTRACT

SOLEY, J.T. & ROBERTS, D.C. 1994. Ultrastructure of ostrich (*Struthio camelus*) spermatozoa. II. Scanning electron microscopy. *Onderstepoort Journal of Veterinary Research*, 61:239–246

The three-dimensional structure and size of ostrich sperm is unknown. In this study, the morphology and dimensions of ostrich sperm were determined by scanning electron microscopy of semen samples obtained from sexually mature males during the breeding season. The results indicate that sperm cells of the ostrich are of the sauropsid type characteristic of non-passerine birds and, in general appearance, resemble those of the chicken, turkey, guinea fowl, budgerigar and tinamou. They differ from tinamou sperm, however, in that they do not show a small bump at the tip of the acrosome. Ostrich sperm are shorter (69,6 μm total length) than those of the chicken, turkey and guinea fowl, but longer than those of the budgerigar. A lack of information makes it impossible to compare the dimensions of ostrich sperm with those of other ratites such as the rhea. In ostrich and guinea fowl, the sperm head is proportionately longer than that of the chicken, turkey and budgerigar as determined by tail to head ratios. Two distinct groups of ostriches could be distinguished on the basis of differences in the length of various sperm cell components. This may reflect persistent genetic (subspecies) variations in the domestic ostrich population.

INTRODUCTION

Scanning electron microscopy (SEM) has been successfully employed to elaborate the three-dimensional structure of the sperm of a number of non-passerine birds, including the chicken (Thurston & Hess 1987), turkey (Marquez & Ogasawara 1975; Thurston & Hess 1987), guinea fowl (Thurston, Hess, Hughes & Froman 1982; Thurston & Hess 1987), budgerigar (Samour, Smith, Moore & Markham 1986), white-naped crane (Phillips, Asa & Stover 1987) and tinamou (Asa, Phillips & Stover 1986). Because of its superior resolution this technique has also been used to accurately determine the dimensions of the various components of sperm cells from some non-passerine birds (Marquez & Ogasawara 1975; Thurston & Hess 1987; Samour *et al*. 1986). In this paper, the three-dimensional structure and size of ostrich sperm is compared with that of other non-passerine birds.

MATERIALS AND METHODS

Semen samples were collected from ten sexually mature male ostriches from the Oudtshoorn district, Cape Province, South Africa, by digital massage of the deferent duct papillae (Berens von Rautenfeld 1977; Bertschinger, Burger, Soley & De Lange 1992). A portion of the ejaculate was fixed overnight at 4 °C in 4 % glutaraldehyde in Millonig's phosphate

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Received 7 July 1994—Editor

buffer. For SEM, glutaraldehyde-fixed sperm suspended and diluted in Millonig's phosphate buffer or phosphate-buffered saline (PBS) (Sanders, Alexander & Braylan 1975) were layered onto poly-L-lysine-coated glass cover slips, freshly cleaved mica sheets, or 0,2 μm Nuclepore filters. The poly-L-lysine was prepared in PBS as described by Sanders *et al.* (1975). The sperm were subsequently dehydrated through a graded ethanol series (25%, 50%, 75%, 96%, 100% x 2–10 min/step) and critical point dried via carbon dioxide in a Polaron E3000 CPD. The cover slips, mica sheets or filters were attached to aluminium stubs with Japan Gold, earthed with Silver Dag, and sputter coated with a thin layer of carbon followed by gold (4,5 min at 10 mA) in a Polaron E5100 coating unit. The samples were viewed in a Philips XL 20 scanning electron microscope operated at 10 kV.

SEM was also employed for the morphometric analysis of the ostrich sperm as this particular technique most accurately portrayed the various components of the sperm cells. Measurements of the acrosome, nucleus, midpiece, principal-piece and end-piece were taken of 20 sperm chosen at random from each of the ten birds. The measurements were made by means of the integrated image analysis system available on the Philips XL 20 scanning electron microscope, after calibration of the system with a calibration grid.

The measurements were statistically analyzed on a desktop computer by means of the Statgraphics (version 6.0, Manugistics Inc. and Statistical Graphics Corporation, 1992) program. For each bird descriptive statistics (mean, standard deviation, etc.) were generated for the acrosome, nucleus, total head length, midpiece, principal-piece, end-piece, total tail length, total length and tail to head ratio using the measurements taken of the 20 sperm for that particular bird. Descriptive statistics were also generated for each sperm cell component (e.g. acrosome, nucleus, etc.) using the measurements taken of the 200 sperm of all the birds. The standardized skewness and kurtosis of each data set were then checked to determine whether or not this data conformed to a normal frequency distribution. To determine whether significant differences existed between the results obtained for each bird, a one-way analysis of variance (ANOVA) was used to compute the F ratio (the between birds mean square value divided by the within birds mean square value) as well as the significance of this F ratio. Finally, Tukey's honest significant differences multiple range test (at the 99% confidence level) was used to make pairwise comparisons between the ten birds to determine which of the birds, if any, produced morphometrically significantly different sperm to the rest of the birds.

RESULTS

Sperm morphology

The spermatozoa were long, narrow, cylindrical structures and displayed a distinct acrosome, nucleus, midpiece, principal-piece and end-piece (Fig. 1). The acrosome formed a short cap-like structure (Fig. 2) which, in damaged sperm, could be seen to cover the tapered anterior aspect of the nucleus (Fig. 3). The longest segment of the sperm head was the nucleus which, in most instances, was gently curved (Fig. 1), although crescent (sickle)-shaped and convoluted forms (Fig. 2) were frequently observed. The nucleus increased progressively in diameter from its junction with the acrosome to its distal termination at the midpiece, with the greatest diameter (0,5–0,6 μm) being reached in the region of the nuclear/midpiece junction. The surface of the nucleus was smooth, but often displayed attached globular bodies (Fig. 3). The relatively short midpiece was slightly wider than the nucleus. The junction between the nucleus and the midpiece was clearly demarcated in most sperm (Fig. 2), although in some instances the transition between the two regions was too gradual to be accurately observed by SEM. The border between the midpiece and the principal-piece was particularly conspicuous and often presented a hooped appearance (Fig. 2). The hoop was considered to represent the annulus observed by transmission electron microscopy (TEM) (Soley 1993). The midpiece

FIG. 1 A complete sperm cell showing the acrosome (A), nucleus (Nu), midpiece (M), principal-piece (P) and end-piece (E). The head is gently curved. SEM X 3000. Bar = 5,0 μm

FIG. 2 An enlarged view of a convoluted sperm head. Note the junctions between the acrosome (A) and the nucleus (Nu) (arrowhead), the nucleus and midpiece (M) (double arrowhead) and the midpiece and the principal-piece (P) (triple arrowhead). SEM X 12500. Bar = 1,0 μm

FIG. 3 The tapered tip of the nucleus (between arrowheads) revealed after loss of the acrosome. Globular particles of proteinaceous material are attached to the main body of the nucleus (Nu). SEM X 17000. Bar = 1,0 μm

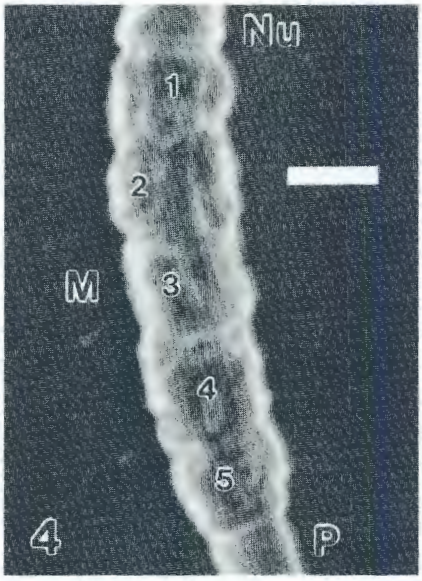
FIG. 4 The midpiece (M) showing the outline of five rows of mitochondria (1–5). Nucleus (Nu), principal-piece (P). SEM X 24500. Bar = 0,5 μm

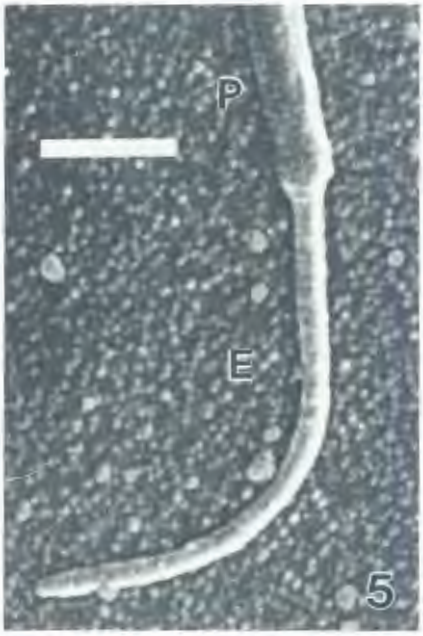
FIG. 5 The narrow end-piece (E) of the tail. Note the abrupt termination of the principal-piece (P). SEM X 17500. Bar = 1,0 μm


A
Nu
M
P
E
1


A
Nu
M
P
2


Nu
3


Nu
1
2
M
3
4
5
P
4


P
E
5

usually appeared smooth but in some sperm the outlines of the mitochondria could be vaguely seen beneath the plasmalemma. Mitochondria could be freely observed only in damaged sperm devoid of the plasmalemma. They were rectangular or polygonal in shape, arranged in a helical pattern around the axoneme, and generally formed five rows along the length of the midpiece (Fig. 4). The principal-piece formed the longest segment of the tail (Fig. 1) and showed no particular surface features, although a circumferential periodicity could sometimes be observed. The junction between the principal-piece and end-piece was clearly defined with the transition between the two regions being either abrupt or gradual (Fig. 5). The short end-piece revealed no specific features.

Morphometric analysis

The lengths of the various segments of the sperm cells of the ten individual ostriches are reflected in Table 1. Descriptive statistics of the sperm cell components of all ten birds combined are shown in Table 2.

In the following description, measurements are given as an arithmetic mean ± standard error.

The acrosome and nucleus displayed a mean length of 1,91 ± 0,02 μm and 10,95 ± 0,08 μm respectively, giving a mean head length of 12,86 ± 0,08 μm. The figures presented for the length of the nucleus are, however, misleading as they do not reflect the tapered portion of the nucleus covered by the acrosome. Nuclear length in this instance therefore reflects only the length of the main body of the nucleus.

The mean length of the midpiece was 3,16 ± 0,02 μm, that of the principal-piece 51,18 ± 0,25 μm, and that of the end-piece 2,39 ± 0,03 μm. The mean total tail length was 56,73 ± 0,26 μm and the mean total length was 69,59 ± 0,31 μm. The tail to head ratio was 4,43 ± 0,03.

The standardized skewness and kurtosis of each data set were found to fall within the range −2,0 to + 2,0, indicating that the data conformed to a normal frequency distribution. One way analysis of variance (ANOVA) of the measurements of the various sperm cell components revealed F ratios significantly ($P < 0,01$) greater than 1, indicating differences between the ten ostriches. Tukey's honest significant differences multiple range test (at the 99 % confidence level) was then used to divide the ten ostriches into homogeneous subgroups based on sperm head length, tail length and total length.

The sperm head length of birds 1, 2, 3, and 4 (13,84 ± 0,09 μm) differed significantly ($P < 0,001$) from that of birds 5, 6, 7, 8, 9 and 10 (12,20 ± 0,08 μm). This increase in head length was chiefly due to an increase in the length of the nucleus (see Table 1).

TABLE 1 Summary of sperm measurements from ten ostriches

Bird no.	Acrosome (μm)	Nucleus (μm)	Total head (μm)	Midpiece (μm)	Principal-piece (μm)	End-piece (μm)	Total tail (μm)	Total length (μm)	Tail to head ratio
1	1,93 ± 0,06	11,60 ± 0,13	13,53 ± 0,14	2,92 ± 0,06	48,22 ± 0,72	2,52 ± 0,11	53,66 ± 0,75	67,19 ± 0,73	3,98 ± 0,08
2	1,95 ± 0,06	11,70 ± 0,18	13,65 ± 0,19	3,44 ± 0,09	55,30 ± 0,41	3,00 ± 0,08	61,74 ± 0,40	75,39 ± 0,42	4,54 ± 0,07
3	1,77 ± 0,04	12,32 ± 0,15	14,09 ± 0,16	3,08 ± 0,07	55,70 ± 0,53	2,39 ± 0,10	61,17 ± 0,58	75,26 ± 0,67	4,35 ± 0,05
4	2,05 ± 0,06	12,04 ± 0,17	14,09 ± 0,18	3,08 ± 0,08	54,11 ± 0,46	2,58 ± 0,06	59,77 ± 0,49	73,86 ± 0,57	4,24 ± 0,06
5	1,81 ± 0,04	10,52 ± 0,13	12,33 ± 0,13	3,13 ± 0,09	51,09 ± 0,67	2,28 ± 0,10	56,50 ± 0,72	68,83 ± 0,78	4,59 ± 0,06
6	1,80 ± 0,04	10,02 ± 0,26	11,82 ± 0,28	3,15 ± 0,07	47,05 ± 0,31	2,27 ± 0,08	52,47 ± 0,33	64,29 ± 0,42	4,48 ± 0,10
7	1,86 ± 0,06	10,13 ± 0,18	11,99 ± 0,18	3,06 ± 0,05	49,36 ± 0,39	2,04 ± 0,05	54,46 ± 0,41	66,45 ± 0,40	4,57 ± 0,09
8	1,91 ± 0,04	10,78 ± 0,20	12,69 ± 0,21	3,25 ± 0,05	49,11 ± 0,33	2,38 ± 0,07	54,74 ± 0,33	67,43 ± 0,40	4,34 ± 0,07
9	1,97 ± 0,04	10,22 ± 0,11	12,19 ± 0,12	3,22 ± 0,04	50,72 ± 0,31	2,20 ± 0,04	56,14 ± 0,30	68,33 ± 0,34	4,61 ± 0,05
10	2,02 ± 0,04	10,17 ± 0,12	12,19 ± 0,11	3,24 ± 0,05	51,14 ± 0,47	2,25 ± 0,05	56,63 ± 0,46	68,82 ± 0,43	4,65 ± 0,07
All 10 birds	1,91 ± 0,02	10,95 ± 0,08	12,86 ± 0,08	3,16 ± 0,02	51,18 ± 0,25	2,39 ± 0,03	56,73 ± 0,26	69,59 ± 0,31	4,43 ± 0,03

Note 1. Sample size for each ostrich = 20
2. Results given as arithmetic mean ± standard error

TABLE 2 Sperm measurement statistics for all ten ostriches combined

Statistics	Acro-some	Nucleus	Total head	Mid-piece	Principal-piece	End-piece	Total tail	Total length	Tail to head ratio
Sample size	200	200	200	200	200	200	200	200	200
Mean (μm)	1,91	10,95	12,86	3,16	51,18	2,39	56,73	69,59	4,43
Standard deviation (μm)	0,23	1,11	1,15	0,32	3,52	0,43	3,73	4,37	0,37
Standard error (μm)	0,02	0,08	0,08	0,02	0,25	0,03	0,26	0,31	0,03
Minimum value (μm)	1,44	8,52	10,29	2,15	42,34	1,45	48,01	60,63	3,32
Maximum value (μm)	2,50	13,63	15,79	3,99	59,05	3,87	65,06	79,11	5,48
Range (μm)	1,06	5,11	5,50	1,84	16,71	2,42	17,05	18,48	2,16
Coefficient of variation (%)	11,87	10,08	8,94	10,23	6,87	17,83	6,58	6,27	8,37

The sperm tail length of birds 2, 3, and 4 (60,89 ± 0,30 μm) differed significantly ($P < 0,001$) from that of birds 1, 5, 6, 7, 8, 9 and 10 (54,95 ± 0,22 μm), and was primarily due to an increase in the length of the principal-piece (see Table 1). The total sperm length of birds 2, 3 and 4 (74,83 ± 0,33 μm) differed significantly ($P < 0,001$) from the total sperm length of birds 1, 5, 6, 7, 8, 9 and 10 (67,33 ± 0,23 μm). Owing to the relatively proportionate increase in length of the chief components of both the head (the nucleus) and the tail (the principal-piece) of longer sperm, the tail to head ratio remained fairly constant for all birds except bird 1. Sperm from this bird displayed a proportionately longer head than the others as shown by a significantly ($P < 0,001$) different tail to head ratio of 3,98 ± 0,08 when compared to that (4,49 ± 0,03) of the other nine birds. These data therefore indicate that birds 2, 3 and 4 belong to one subgroup and birds 5, 6, 7, 8, 9 and 10 to another, with bird 1 displaying some properties of both subgroups.

DISCUSSION

It should be emphasized that a lack of comparative data makes it difficult to compare the dimensions of ostrich sperm statistically with those of other non-passerine birds. The following discussion at best describes a trend or tendency and not statistical differences.

The sperm cells of the ostrich are of the simple (sauropsid) type characteristic of non-passerine birds (Humphreys 1972). In general appearance they resemble those of the chicken, turkey, guinea fowl, budgerigar and tinamou, as detailed by scanning electron microscopy. They differ from tinamou sperm, however, in that they do not show a small bump at the tip of the acrosome. The acrosome of ostrich sperm (1,91 μm) differs very little in length from that of the chicken (≥ 2,0 μm) (Thurston & Hess 1987), duck (2,2 μm) (Maretta 1975a), turkey and guinea fowl (1,6–1,8 μm) (Thurston & Hess 1987) and domestic goose (1,64 μm) (Scheller 1989, cited by Ferdinand 1992), although it is significantly longer than that of the budgerigar (1,42 μm) (Samour *et al.* 1986).

The nucleus of ostrich sperm is smooth in appearance, unlike the rough surface described for chicken, turkey and guinea fowl sperm by Thurston & Hess (1987), although globular units, which probably represent constituents of the seminal plasma, are often seen to adhere to the nuclear surface. The nuclear length of ostrich sperm (10,95 μm) falls within the range reported for the chicken, guinea fowl and duck (see Table 3), but is longer than that observed in the turkey (7–9 μm) (Thurston & Hess 1987), budgerigar (8,0 μm) (Samour *et al.* 1986) and domestic goose (6,82 μm) (Scheller 1989, cited by Ferdinand 1992). Transmission electron microscopy reveals that in chicken, turkey and guinea fowl sperm, the acrosome covers a short projection of chromatin emanating from the anterior aspect of the nucleus (Thurston & Hess 1987). A similar situation is observed in ostrich sperm although the amount of chromatin enclosed by the acrosome is far more substantial (see Fig. 3). In contrast, the acrosomal cap of budgerigar sperm ends adjacent to the nuclear chromatin (Samour *et al.* 1986). Nuclear length, therefore, as measured by SEM, is slightly inaccurate when applied to chicken, turkey and guinea fowl sperm, and misleading in the case of ostrich sperm.

The midpiece of ostrich sperm is similar in length (3,16 μm) to that of the rhea (Phillips & Asa 1989) and tinamou (Asa *et al.* 1986), but appreciably shorter than that of the chicken, turkey, guinea fowl

TABLE 3 Comparative dimensions of some non-passerine bird sperm

Species	Acrosome (μm)	Nucleus (μm)	Head length (μm)	Mid-piece (μm)	Principal-piece (μm)	End-piece (μm)	Tail length (μm)	Total length (μm)	Tail to head ratio	Technique	Reference
Chicken	≥ 2,0	10–14	[14,0][a]	–	–	–	≥ 70,0	> 90,0	[5]	SEM	Thurston & Hess (1987)
	–	–	14,0	4,0	–	2,0	–	> 100,0		TEM[b]	Grigg & Hodge (1949)
	1,5	10,7	[12,2]	2,5	70,0[c]	–	[72,5]	[84,7]	[6]	TEM[b]	Bonadonna (1954)
	2,5	12,5	[15,0]	4,3	90,0[c]	–	[94,3]	[109,3]	[6,3]	TEM	Lake *et al.* (1968)
Turkey	1,6–1,8	7–9	[9,7]	–	–	–	60–65	[73,0]	[6,5]	SEM	Thurston & Hess (1987)
	1,8 (1–2,6)	9,1 (7,2–11)	[11,0]	4,8 (4–6)	61,0[c]	–	[65,8]	76,7	[6]	SEM	Marquez & Ogasawara (1975)
Guinea fowl	1,6–1,8	10–14	[13,7]	–	–	–	60-65	[76,2]	[4,6]	SEM	Thurston & Hess (1987)
	1,8	12,8	[14,6]	3,9	59,0[c]	–	[63,0]	[78,0]	[4,3]	SEM/TEM	Thurston *et al.* (1982)
Duck	2,2	10–11	[12,7]	3–4	–	–	–	–		TEM	Maretta (1975a, 1975b)
Budgerigar	1,42 ± 0,16	8,0 ± 1,97	[9,4]	–	–	–	54,31 ± 5,97	[63,7]	[5,8]	SEM/TEM	Samour *et al.* (1986)
Tinamou	–	–	–	3,0	–	–	–	–		SEM/TEM	Asa *et al.* (1986)
Rhea	–	–	–	3,0	–	–	–	–		TEM	Phillips & Asa (1989)
Ostrich	1,91	10,95	12,86	3,16	51,18	2,39	56,73	69,59	4,4	SEM	Present study

[a] Figures in square brackets have been extrapolated and are not mentioned in the reference
[b] Shadow casting and negative staining of whole mounts
[c] Assumed to include the end-piece

and duck (see Table 3). The observation that the mitochondria of the midpiece can be clearly resolved only when the plasmalemma is absent (Thurston & Hess 1987), was also confirmed in this study. The principal-piece typically forms the longest segment of the sperm cell, and in the ostrich is similar in length to that of the domestic goose (48,61 μm) (Scheller 1989, cited by Ferdinand 1992), but shorter than that of the chicken, turkey and guinea fowl (see Table 3). The circumferential periodicity sometimes observed along this segment of the tail is interpreted as the ribs of the fibrous sheath demonstrated by TEM (Soley 1993). According to Thurston & Hess (1987) the junction between the principal-piece and the end-piece cannot be distinguished by SEM in chicken, turkey and guinea fowl sperm. This junction is clearly demarcated in ostrich sperm, and either a gradual or an abrupt transition between these two segments of the tail can be demonstrated. It must be concluded, therefore, that in the other species examined, the transition is too gradual to be obvious. The length of the ostrich sperm end-piece (2,39 μm) is similar to that recorded in the chicken (2,0 μm) by Grigg & Hodge (1949). The wide range in the measurements of the various segments of ostrich sperm (see Tables 1 and 2) has also been noted in other birds, both at the electron (Marquez & Ogasawara 1975) and light (Wakely & Kosin 1951; McFarlane 1963) microscopical levels.

Although the non-passerine bird sperm examined by SEM are similar in appearance, it has been recognized that they differ in length (Thurston & Hess 1987). Ostrich sperm are similar in length (69,59 μm) to those of the domestic goose (67,41 μm) (Szumowski 1960, cited by Ferdinand 1992), shorter than those of the chicken, turkey and guinea fowl, but longer than those of the budgerigar (see Table 3). Chicken sperm are by far the longest of those of the species investigated, although sperm of the Hawaiian gander and mallard drake are reported to measure approximately 100,0 μm (Humphreys 1972) and those of the pigeon 165,8 μm (Szumowski, Theret & Denis 1976), when viewed with the light microscope. It has been noted, however, that light microscopic measurements are generally greater than those for SEM owing to the high degree of shrinkage associated with the latter technique (Van der Horst, Curry, Kitchin, Burgess, Thorne, Kwiatkowski, Parker & Atherton 1991). A lack of information makes it impossible to compare the dimensions of ostrich sperm with those of other ratites such as the rhea. In ostrich and guinea fowl sperm (Thurston *et al.* 1982; Thurston & Hess 1987) the head is proportionately longer than that of chicken, turkey and budgerigar sperm, as reflected by their tail to head ratios. The tail to head ratios for the ostrich and guinea fowl are 4,4 and 4,6 respectively, while those for the chicken, turkey and budgerigar sperm are 6,3, 6,5 and 5,8 respectively (see Table 3).

The observation that some ostriches produced sperm of significantly different size, poses some interesting questions which merit closer scrutiny. A number of papers have stressed the value of using structural features and morphometric data to distinguish between the sperm of closely related species (McFarlane 1963; Hartley, De Villiers & Hodgson 1985; Visser & Van der Horst 1987; Van der Horst *et al.* 1991). Studies on sperm of the sandhill crane (*Grus canadensis*) revealed a significant positive correlation between an increase in mean sperm head length and fertility. The same study also demonstrated significant differences in sperm head length between four subspecies of sandhill crane (Sharlin, Shaffner & Gee 1979). Data was not available in the present study to allow a comparison between sperm head length and fecundity parameters such as fertility, hatchability and viability. It is interesting, however, to speculate on sperm component length as a taxonomic indicator. As many as six ostrich subspecies or races have been recognized, namely, *Struthio camelus camelus, S.c. molybdophanes, S.c. massaicus, S.c. australis, S.c. spatzi* (apparently now merged with *S.c. camelus*) and the extinct *S.c. syriacus* (Brown, Urban & Newman 1982; Rutgers & Norris 1970). The domesticated South African ostrich on which this study was based is recognized as a hybridized bird descended from local *S.c. australis* stock crossed with North African (*S.c. camelus*) and Syrian (*S.c. syriacus*) birds. This selective crossbreeding occurred in the Cape Province as early as 1876 and was primarily aimed at improving feather quality (Smit 1964). As the ostriches used in the present study represented the domestic variety raised in the Oudtshoorn district of the Cape Province, it is possible that the two different ranges of sperm size observed may reflect persistent genetic (subspecies) variations in the domestic ostrich population.

ACKNOWLEDGEMENTS

The authors are indebted to the Klein Karoo Landboukoöperasie and the University of Pretoria for financial assistance, to Dr W.P. Burger and Dr D.H. de Lange for the collection of semen samples, to Mr H.J. Els for the preparation of material for SEM, to Professors A.J. Bezuidenhout, R.I. Coubrough, G. van der Horst, R.T.F. Bernard and H.J. Bertschinger for critical reading of the manuscript and advice, to Mrs L.B. Oberholzer and Dr P. Turner for assistance with the computer programs, and to the staff of the Oudtshoorn Experimental Farm, in particular Mr C. Nel, for their invaluable help.

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