ELSEVIER

Theriogenology 57 (2002) 1207–1218

Theriogenology

Morphometric characterization and classification of alpaca sperm heads using the Sperm-Class Analyzer® computer-assisted system

P. Buendía[a], C. Soler[a,*], F. Paolicchi[b], G. Gago[a], B. Urquieta[c], F. Pérez-Sánchez[d], E. Bustos-Obregón[e]

[a]*Department of Animal Biology, València University, Burjassot 46100, Spain*
[b]*Instituto Nacional de Tecnología Agropecuaria, Balcarce, Mar del Plata National University, Mar del Plata, Argentina*
[c]*Faculty of Cattle Sciences and Veterinary Medicine, Chile University, Santiago, Chile*
[d]*Parasitology and Cellular Biology Departament, València University, Burjassot, Spain*
[e]*Program of Morfology, Medicine Faculty, Chile University, Santiago, Chile*

Received 30 May 2000; accepted 15 May 2001

Abstract

Sperm morphology has been identified as one characteristic which can be useful in the prediction of sperm fertility, therefore, we hope that this study aimed at establishing standardized morphological criteria might serve in future studies dealing with the search for sperm parameters which facilitate an estimation of sperm quality. For this purpose, ejaculates from fertile alpacas were used to evaluate sperm head morphometry by means of the Sperm-Class Analyzer® (SCA) computer-aided image analysis system. We defined three morphological categories according to sperm head size (normal 50%, small 26%, large 24%) and five categories according to sperm head shape (normal 47%, pyriform 3%, short 20%, round 1%, long 29%). Sperm classification according to shape was performed by first morphometrically characterizing sperm heads clearly falling into each of the shape categories. Thereafter, discriminant analysis was performed on the data from these typical sperm heads and the resulting classification functions were used to categorize 2200 spermatozoa from 11 alpacas. Classification of sperm heads by this method agreed in 88% of the cases with most of the misclassifications being due to pyriform heads classified as long heads. Morphometric values obtained from samples of 50, 100, 150, 175 and 200 sperm heads were compared. At least 150 sperm heads should be evaluated to overcome sample size influence on sperm measurements. Significant differences in sperm morphometry were found between individuals (CV for morphometric parameters ranging from 1.3 to 13.0) and there were marked differences in the sperm morphological composition of the ejaculates. Within-animal CV ranged from 4.7 to 17.8 thus

* Corresponding author. Fax: +34-963864387.
E-mail address: carles.soler@uv.es (C. Soler).

PII: S0093-691X(01)00724-5

showing the high degree of sperm polymorphism present in the alpaca ejaculate.

Keywords: Spermatozoa; Alpaca; Morphometry; Sperm-Class Analyzer®

1. Introduction

South American domestic camelids are farmed primarily for their high quality fiber and as a source of meat. The alpaca (*Lama pacos*, Linnaeus, 1758) is of particular economic importance for inhabitants of the high Andes, with more than 2 million alpacas as a source of animal protein, clothing and fuel [1]. Additionally, this species has been imported into a number of countries outside South America where they are used for fiber production and as pets [2].

Despite the commercial interest of these camelids, their extensive production has been limited by their low (50–60%) fertility rate [3,4]. For this reason, attempts have been made to improve the success rate of alpaca reproduction [5,6]. However, artificial insemination provides no better results than natural breeding [7]. The fertility of the male has not been fully explored, although previous studies have focused on physical characteristics of the semen [1]. Little attention has been paid to the motility and morphology of alpaca spermatozoa even though differences between repeated collections of individual males have been reported for these two important parameters [8].

Some attempts have been made to standardize the evaluation of sperm head morphology in human semen [9,10] but in most animal species the problem of quantifying a normal morphology remains unresolved [11,12]. This question is particularly important in species, such as the alpaca in which several morphological forms are clearly distinguishable at light microscope level. The aims of the present work were to morphometrically characterize alpaca spermatozoa and to determine different morphological classes using multivariate analysis techniques.

2. Materials and methods

2.1. Collection of semen

This study utilized semen from single complete ejaculates of 11 adult male Huacaya alpacas of proven fertility. After being trained for semen collection, workers collected semen samples from each male twice a week for 3 weeks prior to obtention of the samples used in this study. Samples were collected during a 20 min mating with a receptive female and obtained through an artificial vagina designed for this species, maintaining a temperature of 38 °C and using polyethylene sheaths [13].

2.2. Morphometric analysis

Semen smears were prepared, air dried and stained with Hemacolor (standard kit from Merck, Darmstadt, Germany) and permanently mounted.

Morphometric analysis was performed using the morphometric module of the Sperm-Class Analyzer® (SCA) computer-aided sperm analysis system (Microptic, Barcelona, Spain). The equipment consisted of an Olympus BH-2 microscope with a ×100 bright-field oil immersion objective (plan apochromatic; numerical aperture 1.25). The illumination source was centered, and a green filter (IF 550, Olympus, Tokyo, Japan) was placed over the light source to enhance the contrast of the images to be acquired by the computer. The intensity of the bulb and the gain and offset of the camera were standardized for all samples. A Sony CCD AVC-D7CE video camera (Sony Corporation, Tokyo, Japan) with a ×3.3 photo-ocular was mounted on the microscope. The configuration of the computer system included a PIP-1024 B video digitizer board (Matrox Electronic Systems Ltd., Que., Canada) the sperm image analysis software and a high-resolution assistant monitor Sony Trinitron PVM-1443MD (Sony Corporation, Tokyo, Japan). The array size of the video frame recorder was 512 × 512 × 8 bits, digitized images were made up of 262,144 pixels (picture elements) and 256 grey levels. Resolution of images was 0.15 and 0.11 μm per pixel in the horizontal and vertical axes, respectively.

Analysis of sperm midpiece and tail was not performed. The system detected the boundary of sperm heads and their outlines were displayed as white overlays superimposed on the video image. The quality of the image analysis performance was tested visually by superimposing the digitized, binary sperm-head silhouettes over the original grey image and exact fitting was required for approval between the two images. Partially successful head boundary detections were traced manually by the operator using an editing facility provided by the system.

Computer software allowed four basic measurements of sperm heads to be obtained (area in μm^2, perimeter in μm, length in μm, width in μm) and the system was interfaced with a database program to calculate four nondimensional derived parameters (ellipticity, shape factor 1, shape factor 2 and shape factor 3) (Table 1). Data were stored in computer for further analysis.

2.3. Statistical analysis

Kolmogorov–Smirnov and Levene tests were used to check the normality and homogeneity, respectively, of the data variance. Data did not adjust to a normal distribution, so nonparametric tests were used for all the calculations.

Table 1
Parameters assessed for the morphometric characterization of sperm heads

Basic parameters	Derived parameters	
	Parameter	Formula
Area (A)	Ellipticity	$\|L-W\|/(L+W)$
Perimeter (P)	Shape factor 1 (Sf1)	$(4\pi A/P^2)$
Length (L)[a]	Shape factor 2 (Sf2)	Sf1 × (L/W)
Width (W)[a]	Shape factor 3 (Sf3)	$[\pi(L \times W)/4]/A$

[a] L and W are the largest and the smallest values, respectively, of the Feret diameter measured at angles of 0, 30, 60, 90, 120, 150°. W is not necessarily orthogonal to L.

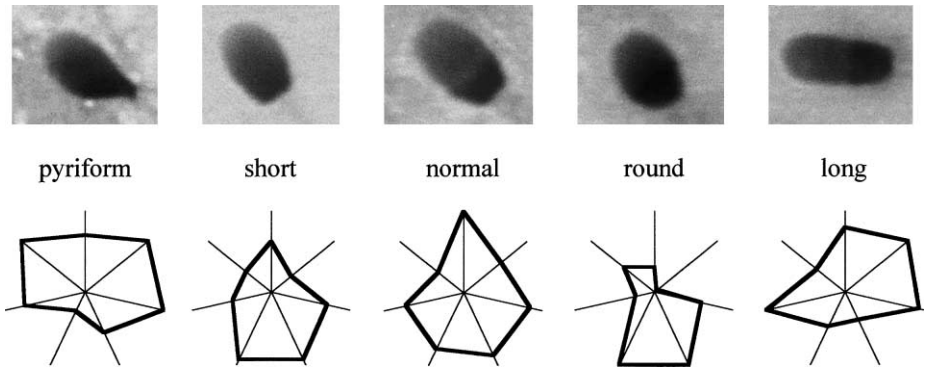

Fig. 1. Representation of one sperm head type of each class and their morphometrical characterization by star glyphs. From the ray at 0° and proceeding counter-clockwise, the rays correspond to the variables: area, ellipticity, length, width, shape factor 1, shape factor 2 and shape factor 3.

To determine the minimum sample size needed to characterize the whole population and the effect-size measure, 200 spermatozoa from each of five animals were analyzed. Subsets of 50, 100, 150, 175 and 200 randomly selected spermatozoa were compared using the Mann–Whitney *U*-test.

Differences between-animals for each morphometrical parameter were evaluated by the Kruskal–Wallis nonparametric test, followed by the a posterior Mann–Whitney *U*-test.

The classification of spermatozoa heads was performed in two steps. The first step consisted of the definition of head size, using the criteria of Bravo et al. [8] to define small, normal and large. The threshold for each class was established on the basis of the area values, considering the 25th percentile small and the 75th percentile large.

The second step concerned the determination of sperm head shape: normal, pyriform, short, round or long (Fig. 1). After defining these classes a number of sperm heads clearly belonging to each one of them were selected and analyzed. Using the data from these spermatozoa, a discriminant analysis was performed using the linear stepwise procedure to identify those parameters that were most useful for the classification of sperm heads into one of the predetermined sperm shape categories. Variables were added to the discriminant functions one by one until we found that adding extra variables did not give significantly better discrimination. The Wilk's lambda [14] was used to compare the fraction of the total dispersion of the data not accounted. The classification matrix obtained after this discriminant analysis was applied to the whole population to establish the proportion of each class present per animal.

The CV was calculated within-animal (estimated as the mean of each individual CV) and between-animals (estimated as the CV of within-animal CV) to establish the best parameters to differentiate among animals on the basis of their sperm morphological parameters.

All statistical analyses were performed with the statistical package for the social sciences version 8.0 for Windows.

3. Results

3.1. Influence of the number of spermatozoa on sperm head values

The results obtained for area, perimeter, length and shape factor 1 showed that 50 spermatozoa were sufficiently representative of the total population since there were no differences when compared with the group of 200 spermatozoa. For other parameters (ellipticity, width and shape factor 3) 100 spermatozoa were necessary to characterize the whole population, given that in two of five animals there were significant ($P < 0.05$) differences between 50 and 200 cells. Finally, 150 spermatozoa were necessary for shape factor 2 because in one individual the group of 100 cells was statistically different ($P < 0.05$) from the 200 cell group. When 100 or more spermatozoa were analyzed, the value of the effect-size measure was not significant ($P > 0.05$).

3.2. Morphometric characterization of alpaca sperm heads

Descriptive statistics of the whole population ($n = 2200$ cells) were calculated to characterize alpaca spermatozoa (Table 2). Values of skewness and kurtosis were not close to zero, indicating that sperm values did not adjust to a normal distribution. Higher CV were observed for ellipticity, area, length and width than for the remaining parameters (perimeter, and shape factors 1, 2 and 3) for which CV were below 9%.

Statistical analysis of morphometric parameters showed differences ($P < 0.05$) between-animals for all the parameters under consideration (Fig. 2).

Within-animal CV ranged from 4.7 (shape factor 1) to 17.8 (ellipticity). Between-animal CV were lower, ranging from 1.3 (shape factor 3) to 13.0 (ellipticity) (Table 3).

3.3. Classification of sperm heads

The classification method used for the establishment of sperm morphological classes did not give significantly better discrimination when perimeter was included, so this parameter was removed from the analysis. The order in which the variables were introduced for the analysis by the program was: ellipticity, shape factors 2 and 1, width, length, shape factor 3

Table 2
Morphometric characterization of alpaca sperm heads

	Area	Perimeter	Ellipticity	Length	Width	Sf1	Sf2	Sf3
Mean	15.95	15.11	0.25	6.10	3.62	0.88	1.48	1.09
S.D.	2.25	1.22	0.06	0.63	0.34	0.05	0.12	0.07
CV(%)	14.11	8.08	24.00	10.33	9.39	5.68	8.11	6.42
Skewness	0.08	−0.05	0.54	0.38	−0.30	−1.22	0.33	0.12
Kurtosis	2.94	2.09	0.80	1.55	1.38	2.98	0.20	1.04
Percentile 25	14.65	14.36	0.22	5.68	3.41	0.85	1.39	1.04
Percentile 75	17.38	15.89	0.29	6.49	3.83	0.91	1.55	1.14

Values of mean and S.D. ($n = 2200$) are given in microns (perimeter, length and width) and squared microns (area) while shape factors and ellipticity are nondimensional.

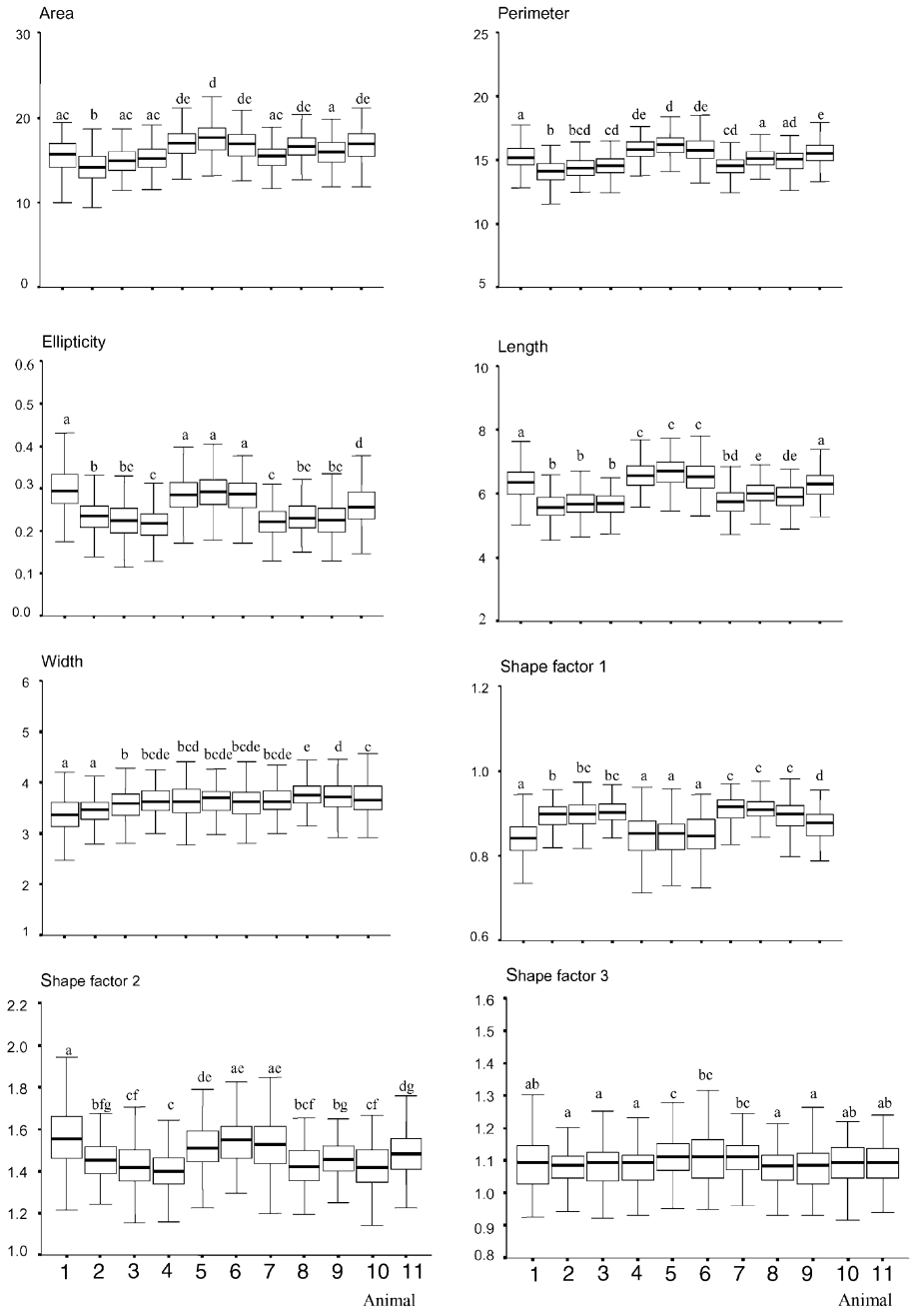

Fig. 2. Box-and-whisker plots showing variations in sperm head morphometric values from animals 1–11 (abscissa). Two hundred spermatozoa were analyzed per animal. Each box encloses the 25th and 75th percentiles, the line in the middle is the median values and the vertical bars extend to 5th and 95th percentiles of the mean values. Boxes labeled with different superscripts are statistically different from each other ($P < 0.05$).

Table 3
Within-animal CV (expressed as the mean of individual values) and between-animal CV

CV	Area	Perimeter	Ellipticity	Length	Width	Sf1	Sf2	Sf3
Within-animal	12.7	6.8	17.8	8.1	8.8	4.7	7.4	6.2
Between-animals	6.5	4.5	13.0	6.7	3.2	3.4	3.8	1.3

and area. Each class was, therefore, defined on the basis of these parameters (Fig. 1). The matrix of classification obtained gave the Fisher's discriminant linear functions for each class (Table 4). This matrix was applied to the reference sperm population with a globally correct assignment of 87.6% of cells. Round, normal and short sperm heads were more accurately classified (more than 90%) than long and pyriform. Most of the misclassifications were observed for these two latter classes (Table 5).

The distribution of spermatozoa in small (area $< 14.65\ \mu m^2$), normal ($14.65\ \mu m^2 <$ area $< 17.38\ \mu m^2$) and large (area $> 17.38\ \mu m^2$) categories showed different percentage values between-animals (Table 6). When the allocation matrix for shape was applied to the whole population, normal cells were the most frequently represented (47.14%), while the most infrequently represented was round spermatozoa (1%). Moreover, differences in

Table 4
Discriminant classification matrix showing Fisher's linear discriminant functions

	Coefficient of function of classification				
	Normal	Pyriform	Short	Round	Long
Area	−268.94	−255.72	−276.69	−273.93	−265.18
Ellipticity	35529.84	35581.53	35084.29	33728.38	35535.30
Length	−138.38	−162.37	−106.34	−80.37	−147.15
Width	2659.42	2578.45	2668.87	2595.46	2639.32
Sf1	13955.28	13964.99	13854.75	13407.72	13912.58
Sf2	−6895.86	−6940.27	−6830.32	−6572.17	−6887.40
Sf3	−3013.29	−2781.35	−3126.29	−3087.17	−2946.71
Constant	−6151.54	−6142.01	−6033.71	−5676.79	−6136.28

Values were obtained from a reference population (normal 93, pyriform 16, short 61, round 6, long 91) by linear stepwise discriminant analysis.

Table 5
Percentage of sperm heads of the reference population assigned to each class

	Number allocated to group					Total
	Normal	Pyriform	Short	Round	Long	
Normal	90.3	0.0	6.5	0.0	3.2	100.0
Pyriform	0.0	56.3	0.0	0.0	43.8	100.0
Short	8.2	0.0	90.2	1.6	0.0	100.0
Round	0.0	0.0	0.0	100.0	0.0	100.0
Long	9.9	2.2	0.0	0.0	87.9	100.0

The 87.6% of the reference sperm population was classificated correctly.

Table 6
Percentage distribution of size and shape classes

Animal	Size			Shape				
	Small	Normal	Large	Normal	Pyriform	Short	Round	Long
1 (188)	31.4	53.7	14.9	20.7	8.5	3.7	1.1	66.0
2 (197)	61.4	34.5	4.1	50.8	4.6	25.3	2.0	17.3
3 (171)	43.3	50.3	6.4	49.7	2.3	35.1	1.8	11.1
4 (176)	38.6	54.0	7.4	49.4	2.8	40.3	1.7	5.8
5 (194)	11.9	46.9	41.2	39.2	6.2	5.1	0.0	49.5
6 (199)	6.5	37.2	56.3	38.2	5.5	2.0	0.0	54.3
7 (185)	12.4	53.5	34.1	32.5	5.4	3.2	0.5	58.4
8 (191)	31.4	53.9	14.7	53.9	0.5	38.2	1.6	5.8
9 (199)	9.0	59.3	31.7	69.4	1.5	24.1	0.0	5.0
10 (193)	22.7	57.0	20.3	53.4	1.0	32.1	2.1	11.4
11 (201)	11.9	51.3	36.8	59.7	0.9	8.0	0.5	30.9
Mean	25.5	50.1	24.4	47.0	3.6	19.7	1.0	28.7

Number of spermatozoa analyzed from each animal are indicated between brackets. No significant statistical differences were found among individuals (Pearson's χ^2, $P > 0.05$).

the distribution of the five shape classes were not found between-animals (Table 6). Sperm heads meeting the criteria of normality for both shape and size represented 24.12% of the whole population.

4. Discussion

Spermatozoa from some species show a constant morphology both intra- and inter-animals (ram [12]; cynomolgus monkey [11]; mouse [15]). By contrast, other species present variation in sperm morphology distribution (human [16,17]; bull [18]; dog [19]). Camelid sperm cells also exhibit high morphological differences [20,21].

This fact has pointed to the need to establish quantitative criteria for the definition of sperm morphology [16,22]. In fact, some effort has been made in this direction, in an attempt to define mammalian spermatozoa based on sperm dimension analysis [23]. Nevertheless, only in the human has systematic work been done [10,24] and reference values are of prognostic interest [25] in regard to fertility.

The subjective evaluation of sperm morphology lacks replication, and the CV associated with this analysis are very high [9,26,27]. This fact has led to the development of computer-assisted semen analysis systems designed for human semen [28–30]. These systems have also been a powerful tool for animal sperm studies and a number of works have been published on this topic for several species [19,31–34].

What constitutes a normal spermatozoon needs to be redefined for most farm animals. For example, in the ram, where more than 95% of the sperm cells are considered normal, significant differences were observed between-animals for most of the morphometric parameters studied [12]. It is not reasonable to ignore this fact in characterizing the reproductive quality of males, considering that some studies have pointed out that

morphometrical values of sperm cells are related to fertility levels for some species [35–38]. Nevertheless, this knowledge has not been used to the same advantage for all species. This is the case of South American camelids, for which information about this approach is very sparse.

As more basic information is collected about factors regulating the reproductive processes in alpacas, it should be possible to improve fertility through better management practices and through the application of reproductive technology. In South America, where most species are seasonal breeders, male alpacas are mated intensively for 2–3 months. During this period, one male may be used to mate many females. This fact discloses how important it is to have a good definition for the male reproductive quality [39].

While measuring sperm head size could be considered an easy task, shape evaluation is completely subjective and commonly expressed in descriptive terms. The SCA® morphometry system was used in this study to calculate and quantify dimensional measurements and express shape irregularities in alpaca spermatozoa. The accuracy and precision of the computer-assisted system used here have been evaluated for several species. The results have yielded low intrinsic CV and high repeatability [12,15,30,40–42]. In the present study, the SCA® system has also been validated for analyzing alpaca sperm. Unfortunately, we have not found any previous reference to alpaca sperm dimensions, and therefore it is not possible to compare our results. Compared to *Camelus dromedarius*, the only camelid for which we have information [43], the spermatozoa of *Lama pacos* seem to be larger both in length (6.10 μm versus 5.62 μm) and in width (3.62 μm versus 2.92 μm).

During the first step of sperm morphometric characterization, it is fundamental to establish which are the most representative parameters for each particular species. In rabbit ejaculate, the sperm head width/length ratio seems to be sufficient for discrimination between-animals [32]. The most suitable parameters for use in the identification of individual rams may be perimeter and shape factor 1 [12]. In the present work, the results resembled those observed in the cynomolgus monkey, where perimeter and shape factors may be the most important parameters [11].

In some species it was possible to differentiate between individuals using CV within- and between-animals, since within-animal CV presented low values, indicating a high constancy of sperm morphometric parameters of an individual, while between-animal CV were relatively high [11,12,40]. In these cases, 100 spermatozoa were sufficient for the morphometric characterization of a semen sample, while in the present study almost 150 sperm cells were necessary. Regarding within-animal CV, similar results were obtained in human semen where intra-individual variation is very high, but in alpaca samples, the variation between-animals was considerably lower than in human samples [10].

The use of multivariate statistical methods, including discriminant and cluster analysis, to define sperm morphological classes has been used in humans [16,30], monkeys [44] and stallions [37]. This is a very useful and powerful statistical tool for determining which parameters and sperm classes should be considered in the morphometrical evaluation of the reproductive quality of species with a high variation in the spermatozoa present in the semen. Moreover, the classification matrix obtained after the analysis permits the classification of new sperm populations, making the continuous study of the reproductive status of animals easy.

The results presented here show significant differences between single ejaculates from different males both in morphometric values and in distribution of sperm classes. This fact was also observed in species, such as the ram, although the general sperm morphology seems to be highly conservative, indicating that it should be possible to define different fertility levels among animals based on the morphometrical evaluation of semen. It has been previously pointed out that in some species there is good correlation between morphology and fertility. Based on the present results, we hope that continued research will be useful in defining the reproductive quality of male alpacas.

Acknowledgements

We thank Dr. P.M. Medina for statistical advice and I.V. Costello for linguistic assistance. This work was partially supported by a travel grant from the Universitat de València to C. Soler.

References

[1] Garnica J, Achata R, Bravo PW. Physical and biochemical characteristics of alpaca semen. Anim Reprod Sci 1993;32:85–90.
[2] Davis GH, Dodds KG, Moore GH, Bruce GD. Seasonal effects on gestation length and birth weight in alpacas. Anim Reprod Sci 1997;46:297–303.
[3] Fernandez-Baca S, Madden DHL, Novoa C. Effect of different mating stimuli on introduction of ovulation in the alpaca. J Reprod Fertil 1970;22:261–7.
[4] Fernandez-Baca S. Manipulation of reproductive functions in male and female New World camelids. Anim Reprod Sci 1993;33:307–23.
[5] Paolicchi F, Urquieta B, Del Valle L, Bustos-Obregón E. Biological activity of the seminal plasma of alpacas stimulus for the production of LH by pituitary cells. Anim Reprod Sci 1999;54:203–10.
[6] San-Martin M, Copaira M, Zuniga J, Rodriguez R, Bustinza G, Acosta L. Aspects of reproduction in the alpaca. J Reprod Fertil 1968;16:395–9.
[7] Bravo PW, Flores U, Garnica J, Ordoñez C. Collection of semen and artificial insemination of alpacas. Theriogenology 1997;47:619–26.
[8] Bravo PW, Flores D, Ordoñez C. Effect of repeated collection on semen characteristics of alpacas. Biol Reprod 1997;57:520–4.
[9] Cooper TG, Atkinson AD, Nieschlag E. Experience with external quality control in spermatology. Hum Reprod 1999;14:765–9.
[10] Ombelet W, Menkveld R, Kruger TF, Steeno O. Sperm morphology assessment: historical review in relation to fertility. Hum Reprod Update 1995;1:543–57.
[11] Gago C, Pérez-Sánchez F, Yeung CH, Tablado L, Cooper TG, Soler C. Standardization of sampling and staining methods for the morphometric evaluation of sperm heads in the cynomolgus monkey (*Macaca fascicularis*) using computer-assisted image analysis. Int J Androl 1998;21:169–76.
[12] Sancho M, Pérez-Sánchez P, Tablado L, de Monserrat JJ, Soler C. Computer-assisted morphometric analysis of ram sperm heads: evaluation of different fixative techniques. Theriogenology 1998;50: 27–37.
[13] Urquieta B, Paolicchi F, Malik de Tchara G, Ferré L, Alberio R. Recolección y caracterización de semen en llama (*Lama glama*). Resúmenes XV Reunión Asociación Latinoamericana Investigaciones Reproducción Humana (ALIRH). Perú: Cuzco, 1997. p. 166–7.
[14] Krzanowski WI. Principles of multivariate analysis. A user's perspective. New York: Oxford University Press, 1988.

[15] Tablado L, Pérez-Sánchez F, Núñez J, Núñez M, Soler C. Effects of exposure to static magnetic fields on the morphology and morphometry of mouse epididymal sperm. Bioelectromagnetics 1998;19:377–83.
[16] Moruzzi JF, Wyrobek AJ, Mayall BH, Gledhill BL. Quantification and classification of human sperm morphology by computer-assisted image analysis. Fertil Steril 1988;50:142–52.
[17] Pérez-Sánchez F, de Monserrat JJ, Soler C. Morphometric analysis of human sperm morphology. Int J Androl 1994;17:248–55.
[18] Gravance CG, Vishwanath R, Pitt C, Casey PJ. Computer automated morphometric analysis of bull sperm heads. Theriogenology 1996;46:1205–15.
[19] Dahlbom M, Andersson M, Vierula M, Alanko M. Morphometry of normal and teratozoospermic canine sperm heads using an image analyzer: work in progress. Theriogenology 1997;48:687–98.
[20] Rai AK, Sharma N, Manivannan B, Khanna ND. Camel semen during breeding and non-breeding seasons. Ind J Anim Sci 1997;67:397–9.
[21] Von Baer L, Hellemann C. Semen variables in llama (*Lama glama*). Arch Med Vet 1998;30:171–6.
[22] Katz DF, Overstreet JW, Samuels SJ, Niswander PW, Bloom TD, Lewis EL. Morphometric analysis of spermatozoa in the assessment of human male fertility. J Androl 1986;7:203–10.
[23] Cummins JM, Woodwall PF. On mammalian sperm dimensions. J Reprod Fertil 1985;75:153–75.
[24] Davis RO, Gravance CG. Consistency of sperm morphology classification methods. J Androl 1994;15:83–91.
[25] World Health Organization. WHO laboratory manual for the examination of human semen and sperm-cervical mucus interaction. 4th ed. Cambridge: Cambridge University Press, 1999.
[26] Davis RO, Gravance CG, Casey PJ. Automated morphometric analysis of stallion spermatozoa. Am J Vet Res 1993;54:1808–11.
[27] Franken DR, Smith M, Menkveld R, Kruger TF, Sekadde-Kigondu C, Mbizvo M, Akande EO. The development of a continuous quality control programme for strict sperm morphology among sub-Saharan African laboratories. Hum Reprod 2000;15:667–71.
[28] Davis RO, Bain DE, Siemers RJ, Thal DM, Andrew JB, Gravance CG. Accuracy and precision of the Cell Form. Human automated sperm morphometry instrument. Fertil Steril 1992;58:763–9.
[29] Kruger TF, Du Toit TC, Franken DR, Acosta AA, Oehninger SC, Menkveld R, Lombard CJ. A new computerized method of reading sperm morphology (strict criteria) is as efficient as technician reading. Fertil Steril 1993;59:202–9.
[30] de Monserrat JJ, Pérez-Sánchez F, Tablado L, Soler C. The Sperm-Class Analyzer®: a new automated system for human sperm morphometry and classification. Contracept Fertil Sex 1995;23(Suppl. 9):S126 [Abstract p. 238].
[31] Ball BA, Mohammed HO. Morphometry of stallion spermatozoa by computer-assisted image analysis. Theriogenology 1995;49:367–77.
[32] Gravance CG, Davis RO. Automated sperm morphometry analysis (ASMA) in the rabbit. J Androl 1995;16:88–93.
[33] Gravance CG, Lewis KM, Casey PJ. Computer automated sperm head morphometry analysis (ASMA) of goat spermatozoa. Theriogenology 1995;44:989–1002.
[34] Sailer BL, Jost LK, Evenson DP. Bull sperm head morphometry related to abnormal chromatin structure and fertility. Cytometry 1996;24:167–73.
[35] Boyle CA, Khoury MJ, Katz DF, Annest JL, Kresnow M, DeStefano F, Schrader SM. The relation of computer-based measures of sperm morphology and motility to male infertility. Epidemiology 1992;3:239–46.
[36] Casey PJ, Gravance CG, Davis RO, Chabot DD, Liu IKM. Morphometric differences in sperm head dimensions of fertile and subfertile stallions. Theriogenology 1997;47:575–82.
[37] Gravance CG, Liu IKM, Davis RO, Hughes JP, Casey PJ. Quantitation of normal head morphometry of stallion spermatozoa. J Reprod Fertil 1996;108:41–6.
[38] Marnet B, Vietez G, Milhet P, Richoilley G, Lesourd F, Parinaud J. Computer-assisted assessment of sperm morphology: comparison with conventional techniques. Int J Androl 2000;23:22–8.
[39] Bravo PW, Solis P, Ordoñez C, Alarcon V. Fertility of female alpaca: effect of daily consecutive breeding. Anim Reprod Sci 1997;46:305–12.
[40] Gago C, Pérez-Sánchez F, Yeung CH, Tablado L, Cooper TG, Soler C. Morphological characterization of ejaculated cynomolgus monkey (*Macaca fascicularis*) sperm. Am J Primatol 1999;47:105–15.

[41] Pérez-Sánchez F, Tablado L, Soler C. Quantitative changes in sperm head morphology during passage through the male excurrent duct system of the rabbit. Mol Reprod Dev 1998;51:203–9.
[42] Soler C, Pérez-Sánchez F, Schulze H, Bergmann M, Oberpenning F, Yeung C-H, Cooper TG. Objective evaluation of the morphology of human epididymal sperm heads. Int J Androl 2000;23:77–84.
[43] Abdel-Raouf M, El Naggar MA. Reproduction in camels (*Camelus dromedarius*). Part 2. The morphology of the camel spermatozoon. J Vet Sci UAR 1965;2:1–11.
[44] Gago C, Soler C, Pérez-Sánchez F, Yeung CH, Cooper TG. Effect of Cetrorelix™ on sperm morphology during migration through the epididymis in the cynomolgus macaque (*Macaca fascicularis*). Am J Primatol 2000;51:103–17.