Llama and alpaca comparative sperm head morphometric analysis

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Abstract

South American camelids are classified in four different species included in two genera. Llama (*Lama glama* L.) and alpaca (*Vicunya pacos*, L.) are the domestic species, presenting a high economical interest. This classification is based on historical, evolutionary and morphological criteria, but the level of hybridization and the total reproductive compatibility make it difficult to use the "biological criteria" based on reproductive isolation. The aim of the work was to see if there are differences on spermatozoa morphometry indicating a pre-zygotic isolation mechanism based on sperm competition. Nine adult Lanuda (Ch'aku) llamas and eleven Huacaya alpacas were used. After obtaining the samples using an artificial vagina, semen smears were stained with Hemacolor kit. Samples were automatically analysed using the morphometry module of the ISAS®v1 CASA system. Almost 100 sperm cells were analysed per sample. The following parameters were calculated: Length, Width; Perimeter, Area, Ellipticity, Rugosity, Regularity and Shape Factor. Most of the parameters (except rugosity and regularity) were significantly different between both species, being greater in alpaca than in llama. Looking to the variation data, both intra and inter-animal coefficients of variation (CV) were also different, but in this case the variation was higher in both CV in llama than in alpaca. From the point of view of the sperm competition, all these data would suggest that both species are in the process of a gamete isolation process. A good selection of sperm characteristics could help farmers to define the "purity" of each animal using the morphometrical analysis, not much more effective than other techniques, but much simpler and less expensive.

Keywords: Llama, alpaca, sperm morphometry, ISAS, speciation

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Introduction

Following classical taxonomy, the camelids of the New World comprise four species, possessing 37 pairs of chromosomes (Taylor et al., 1968). All of them were originally classified in the same genus Lama (Brown, 2000), but the last classification included two different genera. Following this, the species are: the domestic llama, *Lama glama* (L, 1758) and alpaca, *Vicugna pacos* (L, 1789); and their wild ancestors the guanaco, *Lama guanicoe* (Müller, 1776) and vicuña, *Vicugna vicugna* (Molina, 1782), respectively. In addition there are two species from the Old World, dromedary, *Camelus dromedarius* (L,

1758), and Bactrian camel, *Camelus bactrianus* (L, 1758) (Wheeler, 2012).

South American camelids (SAC) are an important social and economical source for native farmers of the Andean region, providing good quality fiber (alpacas), and packing and meat (llamas) (Wheeler et al., 1995). For this reason it is important to define what each species is in order to establish adequate genetic programs to avoid the high-uncontrolled hybridization levels we can find between them. This must be essential for future genetic programs, including controlled hybridization schemes. This issue is of increasing interest due to recognized potential economic benefits of local genetic resources and the threats that face marginal and extensive agriculture (Hall and Bradley, 1995). Moreover, in recent years there has been an increase in the commercial rearing of SAC away from their original habitat in countries such as Australia, New Zealand and the USA (Brown, 2000).

In the present work sperm morphometry of two species of SAC was compared. Theoretical models assume that a constant amount of resource is available to produce an ejaculation, and that the size of gametes is directly proportional to the resources invested in each. Such models conclude than sperm competition should favour an increase in sperm numbers at the expense of a reduction in size (Parker, 1993). An alternative hypothesis proposes that both sperm numbers and size should increase under sperm competition, because an increase in size results in faster swimming speed (Gomendio and Roldan 1991, 2008; Gomendio et al., 2011). Regarding the animal size in relation to sperm size, it seems that as large as the animal is, as small are his spermatozoa (Cummins and Woodall, 1985; Gomendio et al., 2011). Among large mammals (with low mass-specific metabolic rates), sperm size is influenced by metabolic rate (corrected for body mass) but not by levels of sperm competition (Gomendio et al., 2011). In addition, producing longer sperm takes more time, so that spermatogenesis takes longer in species with longer sperm (Ramm and Stockley, 2010).

On the other hand, across a wide range of species, all sperm components increase in size in response to sperm competition because they all contribute in complementary ways to increase in sperm swimming velocity (Gómez Montoto et al., 2011). Particularly, in relation to head dimensions, an increase in sperm elongation (defined as L/W) could reduce drag and lead to a more efficient propulsion (Malo et al., 2006; Gillies et al., 2009; Tourmente et al., 2011). However, studies on closely related species and intraspecific comparisons have not always revealed a clear association between sperm swimming velocity and sperm size (Gage et al., 2002). This could correspond with the fact that each parameter is being quantified in different subsamples from the same male (Fitzpatrick et al., 2013). Only when it is possible to measure both motility and morphometry in the same cells will we be able to bypass this limitation.

It seems that sperm competition is associated with the area of the sperm head, because it could be argued that such increase in sperm head size may not be solely related to increases in swimming velocity. Also this increase can facilitate interactions and attachment with oviductal epithelial cells and that may also obstruct the attachment of spermatozoa from rival males (Anderson et al., 2006; Gómez Montoto et al., 2011).

Finally, regarding seminal and copulation characteristics, both are

equivalent in llamas and alpacas. This means that from a reproductive point of view both species can compete for the same females, suggesting that sperm competition is possible between males of both species (Brown, 2000).

Previous works have dealt independently with alpaca (Buendia et al., 2002) and llama (Soler et al., 2013). In both cases commercial CASA systems were used to analyse the sperm head morphometry. The aim of the present work was to establish if the sperm head morphometric characteristics of llama and alpaca are equivalent or if we can observe enough differences to postulate a possible gamete evolutive divergence.

Materials and methods

Collection and preparation of the samples

Semen from complete ejaculates of 9 adult male llamas (Lanuda -Ch'aku- breed) and 11 adult male alpacas (Huacaya breed) with proven fertility were used. All animals were involved in a genetic improvement program and can be considered genetically pure specimens. After being trained for semen collection, semen samples were collected from each male twice a week for 3 weeks prior to extraction of the samples used in this study (one sample per male). Samples were collected during a 20-minute mating with a receptive female and obtained through an artificial vagina designed for the species, maintaining a temperature of 38ºC and using polyethylene sheaths (Urquieta et al., 1997). With this system, a male may copulate for a time comparable to that of natural mating, the semen samples are reliable, and the sexual behaviour of males is not modified (Bravo et al., 1997a).

Morphometric analysis

Semen smears were air dried and stained with the Hemacolor kit (Merck, Darmstadt, Germany). Briefly it consists of 5 dips into the fixative (methanol 50%), 7 into the first stain solution and 6 into the second one. After that, the slides were air dried and permanently mounted with Eukkit (o. Kindler GmbH and Co., Freiburg, Germany).

Morphometric analysis was done using the morphology module of the Integrated Semen Analysis System (ISAS®v1), computer assisted equipment (Proiser R+D S.L., Paterna, València, Spain). Slides were examined using a UOP-UB203 (Chongqing, China) microscope equipped with a 100x bright field objective and a 1x photo-ocular. The video signal was acquired by a Proiser 782M video camera (Proiser R+D, Paterna, València, Spain). The array size was 768x576x12 bits providing digitalized images of 442368 pixels and 256 grey levels. The resolution of images was 0.084 µm/pixel in both horizontal and vertical axes.

One hundred sperm cells per sample presenting no overlapping with other cells or with background particles were randomly captured using a software function. Several sperm head parameters of size (length, width, area and perimeter) and shape (regularity, rugosity, ellipticity and shape factor) were measured (Soler et al., 2013).

Statistic Analysis

Normal distributions and variance homogeneity were checked by Kolmogorof-Smirnov and Levene tests, respectively. For parameters with normal distribution and homogeneous variances a one-way ANOVA was performed. For the other parameters the Kruskal-Wallis non-parametric test was done.

All statistical analyses were performed using the SPSS, version 11.5 (SPSS Inc., Chicago, Illinois). Results are presented as average ± SD. Differences were considered significative when $P<0.05$.

Results

Sperm head morphometric comparison between llama and alpaca

Most of the sperm head morphometric and shape parameters were significantly different between llama and alpaca. Sperm heads were larger in alpaca, presenting higher ($p<0.05$) ellipticity, shape factor, but no significant differences for rugosity and regularity (Table 1).

Intra- and inter-animal sperm head morphometry variation in alpacas and llamas

Almost all sperm head morphometry parameters showed larger CVs, both intra- and inter-animal in llama than in alpaca (Table 2). Only the length was similar and ellipticity was lower in llama for inter-animal CVs (Table 2).

Discussion

In biology, a species is one of the basic units of biological classification and a taxonomic rank, but there is not only one definition of what a species is and so the difficulty of defining species is known as the species problem (Mallet, 2007).

In this regard, almost three principal concepts could be considered. The morphological approach is related with similarity of DNA (Vidal-Rioja et al., 1994), morphological, physiological and ecological niche features of a group of populations (Mallet, 2007). This kind of approach was the base for classifying SAC into four different species (Kadwell et al., 2001).

Following the species evolutionary concept criteria, we consider that modern camelids stem from a common ancestor, who appeared and evolved for more than 40 million years (MY) in the North American continent. By late Miocene, the ancestral camelid split into two branches which, after successive diversifications, emigrated to South America and Eurasia about 3 MY ago (Vidal-Rioja et al., 1994). At present, South American native artiodactyls are represented, among others, by the Tylopoda suborder, which comprises two wild forms of camelids, guanaco (*Lama guanicoe*) and vicuña (*Vicugna vicugna*), and their respective domestic derivatives llama (*Llama glama*) and alpaca (*Llama pacos*) (Maté et al., 2004). As we can see in this classification the alpaca is included into the genus Llama and not Vicugna. Anyway, possible crossings influences between the wild species on domestic ones cannot be excluded (Kadwell et al., 2001; Renieri et al., 2009).

A species is often defined following a biological concept as a group of organisms capable of interbreeding and producing fertile offspring. This is the case of the camelids and so, following this criterion all the camelids could be considered only one species. All the SAC maintain the same chromosome number and can be hybridized producing fertile offspring (Kadwell et al., 2002). Moreover, the possibility of establishing a sole connection to llama and/or alpaca species based on genetic "purity" is not sustained because: (i) both species have the same phylogenetic origin, with a great amount of common genes; (ii) the genetic drift caused by the conquest "shock"; (iii) the "dark mixture" between animals of both species; and (iv) the occasional crossing with wild species at some stage in time (Renieri et al., 2009).

Only 20% of alpacas and 50% of llamas remain pure (Kadwell et al., 2002). Recent papers have established a higher hybridization level in alpacas up to 90%, indicating that the original alpaca genome is danger of disappearing (Wheeler 2005, 2012).

Looking to the genetic traits, extensive mitochondrial DNA (mtDNA) sequence analysis of native SAC reveals two distinct genetic groups (halotypes), representing the two divergent evolutionary lineages of the vicugna and guanaco. About 80% of all llama and alpaca sequences fall in the guanaco lineage, and the individuals possessing vicuña-like mtDNA sequences were mostly alpacas or pacovicuña (alpaca/vicuña hybrids). So, mtDNA evidence alone tended to support a guanaco ancestry for both domestic forms, with some hybridisation with vicuña having occurred during or after domestication (Kadwell et al., 2001). In contrast, the use of microsatellite nuclear DNA showed that the llama is more similar to the guanaco, while the alpaca is more similar to the vicuña. The combined microsatellite and mitochondrial DNA analysis produces striking results, which show extensive nuclear introgression in the llama and mostly mitochondrial introgression in the alpaca (Kadwell et al., 2002). So, following this criterion the alpaca could be classified *Vicunga pacos*, instead of the "original" classification into the Llama genus (Kadwell et al., 2001).

Following all that we have exposed, it is quite obvious that the classification of SAC is anything but clear.

A recent paper compares accurately most of the seminal characteristics of llamas and alpacas (Bravo et al., 2013). Llamas and alpacas share the same mating behaviour with a very long sexual intercourse time (10-50 min). Ejaculation is continuous during all this time, without fractions and with a reasonably uniform semen quality from beginning to end (Lichtenwalner et al., 1996; Soler et al., 2013). The seminal characteristics are quite similar, with very viscous plasma forming a coagulum soon after copulation (Bravo et al., 1997b). SAC semen requires a considerable time for liquefaction, in alpaca averaged 23h (Bravo et al., 2000). Regarding the morphology both species showed similar values or normal cells (Bravo et al., 2013). When morphometry of sperm heads was analysed in order to classify the sperm heads into different morphological classes, the results were different between llama and alpaca, using the criterion that the most common class is that considered as normal. So, what was previously considered normal for alpaca (Buendia et al., 2002) was considered long in llama, being the cells named short in alpaca that are normal in llama (Soler et al., 2013). This difference on what class was more frequent contributed to the design of the present work.

It is likely that the fertilization environment that gametes must function within has directly selected the evolution of sperm form and function (Gage et al., 2002). The fertilization environment varies extensively across taxa, but also inside a concrete taxon. In fact, inside species different genetic combinations could result in different characteristics of the female reproductive tract structure and function, and so it could act like a selective pressure to cement characteristics of spermatozoa from different males. This seems to be the case of SAC where, like in other mammals, sperm may be forced to migrate through an elongated reproductive tract with filtration from an immune response and cervical mucous (Katz et al., 1989). There is comparative evidence that the

fertilization environment has a direct influence on spermatozoal evolution: sperm size is associated with the dimensions of the female reproductive tract across a number of taxa (Gage et al., 2002).

The genetic control of sperm morphometry has received little attention. Studies on mice (Ward, 2000), rabbit (Napier, 1961) and zebra finch (Birkhead et al., 2005) sperm showed direct heritability of head and tail components. This inheritance value provides a mechanism for the maintenance of profound variance in sperm morphometry, and could be the base for speciation, and it is the base of what we want to propose in this work. Traits that are closely related to fitness are expected to have lower heritabilities because of stabilizing selection (Mousseau and Roff, 1987). In this sense, morphology tends to have higher heritability than other characters more related with fitness, such as motility (Morrow and Gage, 2001a). On the other hand, sperm size seems to be condition-independent in opposition to motility and/or vitality (Morrow and Gage, 2001b).

It has not been defined if sperm size is subject to stabilizing or directional selection, or both. Sperm competition could select directionally (LaMunyon and Ward, 1999), but the female reproductive tract is an environment likely to generate stabilizing selection and there is evidence for associations between storage site dimensions and sperm size across taxa (Morrow and Gage, 2001a). Much more work is related with comparisons on the values between experimental populations, but also the analysis of the variation is a very valuable analysis. It has been pointed out in the present results that not only the morphometric data but also the variation, both inter and intra animal, was different in both species.

There are doubts about the genetic structure of sperm morphometry. Some midpiece characters seem to present post-meiotic expression (Distel et al., 1984; Oko, 1998), or haploid effects (Johnson et al., 1995). But if haplotypic expression was significant within an ejaculation, very high variation in sperm phenotype within a male was expected. In general, the values referring to sperm morphometry present a narrow distribution that is different from other males in the population, providing evidence that the sperm phenotype is under the singular control of the diploid parent (Morrow and Gage, 2001b). The differences we have found here are not so high as to indicate a different way to express the morphometry of the head in llama and alpaca.

Sperm form and function is likely to experience intensely focused selection at this critical stage in reproduction. So, sperm length must be therefore optimized independently of the male producer (Morrow and Gage, 2001b). We have found different variations both between and within males, being significantly higher in llama than in alpaca, and this could be explained by different temporal or spatial variations in female tract characteristics and/or sperm competition level (Morrow and Gage, 2001b).

In fact, different insect species showed positive associations between female reproductive tract dimensions and sperm size, suggesting co-evolution between female reproductive and male gametic traits (Morrow and Gage, 2001b). We hope for some similar results in our work but the dimensions of the female tract in llamas and alpacas do not differ so much as to explain differences

between head sperm size in these species (Sumar and Adams, 2007).

We have exposed before that all of them are classified as four different species while all of them are able to reproduce with hybrids, being fertile between them and the other individuals of the other species. Nevertheless, the results presented here showed that the morphometric characteristics of sperm heads from alpaca and llama differ significantly. In different species it has been shown that each individual male produces specific population of sperm dimensions, which is significantly different from those of other males in the same population (Gage et al., 1998; Gago et al., 1998; Soler et al., 2005; Gil et al., 2009; Bellastella et al., 2010; Gallego et al., 2012). This was also observed in alpaca (Buendía et al., 2002) and llama (Soler et al., 2013). But in the present work we have found that both intra- and inter- animal CVs were significantly higher in llama than in alpaca. Following what we have exposed before, this seems to indicate that the sperm competition among llama males could be higher than in alpaca. Moreover, we must assume that the probability of inter species fertility must be lower than that corresponding to the intra-species copulation.

Finally, we must return to the species question. Despite SAC are classified traditionally into four different species, animals of all these species can produce hybrids in which reproductive capability remains intact as in the original pure breeds. This fact implies that, from both a biodiversity and agricultural benefit point of view, good definitions of these species are necessary. While there are problems regarding the establishment of species, when we talk about races the dilemma is even deeper. Because different SAC breeds have different phenotypic characteristics (fiber quality, particularly), it is important to define a good map of distribution of breeds, just as it was established in other species (Wheeler et al., 1995; González et al., 2006; Renieri et al., 2009, Wheeler, 2012).

Conclusions

Here we have shown that, when we take well-defined breeds of two of the South American camelids (Huacaya for alpaca and Lanuda -Ch'aku- for llama) they differ in sperm morphometry, indicating that morphometric parameters could result in a simple but significant way to differentiate between them. Future work must be developed considering hybrids of these two species in which it is expected that these traits could present intermediate values. Moreover, guanaco and vicuña must be included to complete a sperm morphometry map of SAC.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

Table 1 Morphometric characterization of Llama and Alpaca sperm heads

Parameter	Alpaca	Llama
Area	15.9±2.3	13.7±2.8*
Perimeter	15.1±1.2	14.1±1.6*
Length	6.1±0.6	5.5±0.7*
Width	3.6±0.3	3.4±0.4*
Rugosity	0.9±0.1	0.9±0.1
Shape factor	1.5±0.1	1.4±0.1*
Regularity	1.1±0.1	1.1±0.1
Ellipticity	0.3±0.1	0.2±0.1*

Values of Mean and S.D. (Alpaca, n=2,200, Llama n=3,207) are given in μm (perimeter, length and width) and μm^2 (area), while derived parameters are no dimensional. *Significantly different between species ($p<0.05$).

Table 2 Intra-animal and inter-animal and total Coefficients of Variation (CV) for sperm morphometric parameters in Llama and Alpaca

Parameter	Species	Intra-animal	Inter-animals	Total
Area	Alpaca	12.7	6.5	14.11
	Llama	18.2	8.2	20.51
Perimeter	Alpaca	6.8	4.5	8.08
	Llama	7.2	8.0	11.36
Length	Alpaca	8.1	6.7	10.33
	Llama	9.0	6.5	12.52
Width	Alpaca	8.8	3.2	9.39
	Llama	9.4	7.1	12.43
Rugosity	Alpaca	4.7	3.4	5.58
	Llama	6.2	9.8	6.70
Shape factor	Alpaca	7.4	3.8	8.11
	Llama	8.3	4.2	9.22
Regularity	Alpaca	6.2	1.3	6.42
	Llama	7.2	7.3	5.61
Ellipticity	Alpaca	17.8	13.0	24.00
	Llama	22.9	6.5	25.00

Intra-animal CV is expressed as the mean of individual values; inter-animal CV of individual CVs; Total CV was calculated considering all the analysed cells for each species (like all the spermatozoa belongs to only one animal from this species)

References

Anderson, M.J., Dixson, A.S., Dixson, A.F., 2006. Mammalian sperm and oviducts are sexually selected: evidence for co-evolution. J. Zool., 270, 682-686.

Bellastella, G., Cooper, T.G., Battaglia, M., Ströse, A., Torres, I., Hellenkemper, B., Soler, C., Sinisi, A.A., 2010. Dimensions of human ejaculated spermatozoa in Papanicolau-stained seminal and swim-up smears obtained from the Integrated Semen Analysis System (ISAS®). Asian J. Androl., 12, 871-879.

Birkhead, T.R., Pellatt, E.J., Brekke, P., Yeates, R., Castillo-Juárez, H., 2005. Genetic effects on sperm design in the zebra finch. Nature, 434, 383-387.

Bravo, P.W., Flores, D., Ordóñez, C., 1997a. Effect of repeated collection on semen characteristics of alpacas. Biol. Reprod., 57, 520-524.

Bravo, P.W., Flores, U., Garnica, J., Ordóñez, C., 1997b. Collection of semen and artificial insemination of alpacas. Theriogenology, 47, 619-626.

Bravo, P.W., Ccallo, M., Garnica, J., 2000. The effect of enzymes on semen viscosity in llamas and alpacas. Small Rum. Res., 38, 91-95.

Bravo, P.W., Alarcón, V., Baca, L., Cuba, Y., Ordóñez, C., Salinas, J., Tito, F., 2013. Semen preservation and artificial insemination in domesticated South American camelids. Anim. Reprod. Sci., 136, 157-163.

Brown, B.W., 2000. A review on reproduction in South American camelids. Anim. Reprod. Sci., 58, 169-195.

Buendía, P., Soler, C., Paollichi, F., Gaco, C., Urquieta, B., Pérez-Sánchez, F., Bustos-Obregón, E., 2002. Morphometric characterizationand classification of alpaca sperm heards using the Sperm-Class Analyzer computer-assisted system. Theriogenology, 57, 1207-1218.

Cummins, J.M., Woodall, P.F., 1985. On mammalian sperm dimensions. J. Reprod. Fertil., 75, 153-175.

Distel, R.J., Kleene, K.C., Hecht, N.B., 1984. Haploid expression of a mouse testis alpha-tubulin gene. Science, 224, 68-70.

Fitzpatrick, J.L., García-González, F., Evans, J.P., 2013. Linking sperm length and velocity: the importance of intramale variation. Biol. Lett., 6, 797-799.

Gago, C., Pérez-Sánchez, F., Yeung, C.-H., Tablado, L., Cooper, T.G., Soler, C., 1998. Standardization of sampling and staining methods for the morphometric evaluation of Cynomolgus monkey (*Macaca fascicularis*) sperm heads using computer-assisted image analysis. Int. J. Androl., 21, 169-176.

Gage, M.J.G., Stockley, P., Parker, G.A., 1998. Sperm morphometry in the Atlantinc salmon. J. Fish Biol., 53, 835-840

Gage, M.J.G., Macfarlane, C., Yeates, S., Shackleton, R., Parker, G.A., 2002. Relationships between sperm morphometry and sperm motility in the Atlantic salmon. J. Fish Biol., 53, 1528-1539.

Gallego, V., Peñaranda, D.S., Marco-Jiménez, F., Mazzeo, I., Pérez, L., Asturiano, J.F., 2012. Comparison of two techniques for the morphometry study on gilthead seabream (*Sparus aurata*) spermatozoa and evaluation of changes induced by cryopreservation. Theriogenology, 77, 1078-1087.

Gil, M.C., García-Herreros, M., Barón, F.J., Aparicio, I.M., Santos, A.J., García-Marín, L.J., 2009. Morphometry of porcine spermatozoa and its functional significance in relation with the motility parameters in fresh semen. Theriogenology, 71, 254-263.

Gillies, E.A., Cannon, R.A., Green, R.B., Pacey, A.A., 2009. Hydrodinamic propulsion of human sperm. J. Fluid Mech., 625, 444-473.

Gomendio, M., Roldan, E.R.S., 1991. Sperm competition influences sperm size in mammals. Proc. Royal Soc. London B, 243, 181-185.

Gomendio, M., Roldan, E.R.S., 2008. Impliations of diversity in sperm size and function for sperm competition and fertility. Int. J. Dev. Biol., 52, 439-447.

Gomendio, M., Tourmente, M., Roldan, E.R.S., 2011. Why mammalian lineages respond differently to sexual selection: metabolic rate constrains the evolution of sperm size. Proc. Royal Soc. London B, 278, 3135-3141.

Gómez Montoto, L., Varea Sánchez, M., Tourmente, M., Martín-Coello, J., Luque-Larena, J.J., Gomendio, M., Roldan, E.R.D., 2011. Sperm competition differentially affects swimming velocity and size of spermatozoa from closely related muroid rodents: head first. Reproduction, 142, 819-830.

González, B.A., Palma, R.E., Zapata, B., Marín, J.C., 2006. Taxonomic and biogeographical status of guanaco *Lama guanicoe* (Artiodactyla, Camelidae). Mammal Rev., 36, 157-178. Hall, S.J.G., Bradley, D.G., 1995. Conserving livestock breed diversity. Trends Ecol. Evol., 10, 267-270.

Johnson, L.R., Pilder, S.H., Bailey, J.L., Oldsclarke, P., 1995. Sperm from mice carrying one or 2 t-haplotypes are deficient in investment and oocyte penetration. Dev. Biol.,168, 138-149.

Kadwell, M., Fernández, M., Stanley, H.F., Baldi, R., Wheeler, J.C., Rosadio, R., Brudford, M.W., 2001. Genetic analysis reveals the wild ancestors of the llama and alpaca. Proc. Royal Soc. London B, 268, 2575-2584.

Kadwell, M., Stanley, H.F., Fernández, M., Wheeler, J.C., Rosadio, R., Baldi, R., Brudford, R., 2002. Genetic analysis and the domestication of the new world camelids. Int. Camelid Quart., 2, 1-3.

Katz, D.F., Drobnis, E.Z .& Overstreeet, J.W., 1989. Factors regulating mammalian sperm migration through the female reproductive tract and oocyte vestments. Gam. Res., 22, 443-469.

LaMunyon, C.W., Ward, S., 1999. Evolution of sperm size in nematodes: sperm competition favours larger sperm. Proc. Royal Soc. London B, 266, 263-267.

Lichtenwalner, A.B., Woods, G.L., Weber, J.A., 1996. Seminal collection, seminal characteristics and pattern of ejaculation in llamas. Theriogenology, 46, 293-305.

Mallet, J. 2007. Species, concepts of. In *Encyclopedia of Biodiversity*: 1-15. Levin, S.A. (Ed). Oxford: Elsevier.

Malo, A.F., Gomendio, M., Garde, J., Lang-Lenton, B., Soler, A.J., Roldan, E.R.S., 2006. Sperm design and sperm function. Biol. Let., 2, 246-249.

Maté, M.L., di Rocco, F., Zambelli, A., Vidal-Rioja, L., 2004. Mitochondrial DNA structure and organization of the control region of South American camelids. Mol. Ecol. Notes, 4, 756-767.

Morrow, E.H., Gage, M.J.G., 2001a. Artificial selection and heritability of sperm length in *Grillus bimaculatus*. Heredity, 87, 356-362.

Morrow, E.H., Gage, M.J.G., 2001b. Consistent significant variation between individual males in spermatozoal morphometry. J. Zool. London, 254, 147-153.

Mousseau, T.A., Roff, D.A., 1987. Natural selection and the heritability of fitness components. Heredity, 59, 147-153.

Napier, R.A.N., 1961. Fertility in the male rabbit III. Estimation of spermatozoan quality by mixed insemination, and the inheritance of spermatozoan characters. J. Reprod. Fert., 2, 273-289.

Oko, R., 1998. Occurrence and formation of cytoskeletal proteins in mammalian spermatozoa. Andrologia, 30, 193-206.

Parker, G.A., 1993. Sperm competition games: sperm size and sperm number under adult control. Proc. Royal Soc. London B, 253, 245-254.

Ramm, S.A., Stockley, P., 2010. Sperm competition and sperm length influence the rate of mammalian spermatogenesis. Biol. Let., 6, 219-221.

Renieri, C., Frank, E.N., Rosati, A.Y., Antonini, M., 2009. Definición de razas en llamas y alpacas. Anim. Gen. Res. Inf., 45, 45-54.

Soler, C., Gadea, B., Soler, A.J., Fernández-Santos, M.R,. Esteso, M.C., Núñez, J., Moreira, P.N., Núñez, M., Gutiérrez, R., Sancho, M., Garde, J.J., 2005. Comparison of three different methods for the assessment of Epididymal red deer sperm morphometry by computerized analysis with ISAS®. Theriogenology, 64, 1236-1243.

Soler, C., Sancho, M., García, A., Fuentes, M.C., Núñez, J., Cucho, H., 2013. Ejaculate fractioning effect on llama sperm head morphometry as assessed by ISAS® CASA system. Reprod. Dom. Anim., 49, 71-78.

Sumar, J., Adams, G.P., 2007. Reproductive anatomy and life cycle of the male and female llama and alpaca. In *Current Therapy in Large Animal Theriogenology* (2nd ed.): 855-865. Youngquist RS, Threlfall WR (Eds). St Louis MO: Saunders Elsevier.

Taylor, K.M., Hungerford, D.A., Snyder, R.L., Ulmer, F.A., 1968. Uniformity of karyotypes in the Camelidae. Cytogenetics, 7, 8-15.

Tourmente, M., Gomendio, M., Roldan, E.R.S., 2011. Sperm competition and the evolution of sperm design in mammals. BCM Evol. Biol., 11, 12.

Urquieta, B., Paolicchi, F., Malik de Tchara, G., Ferré, L., Alberio, R. ,1977. Recolección y caracterización de semen en llama (*Lama glama*). *XV Reunión ALIRH*. 166-167.

Vidal-Rioja, L., Zambelli, A., Semorile, L., 1994. An assessment of the relationships among species of Camelidae by satellite DNA comparisons. Hereditas, 121, 283-290.

Ward, P.I., 2000. Sperm length is heritable and sex-linked in the yellow dung fly (*Scathophaga stercoraria*). J. Zool., 251, 349-353.

Wheeler, J.C., 2005. Camelid research in Peru. Int. Camelid Quart. Mag., 4, 7-10.

Wheeler, J.C., 2012. South American camelids – past, present and future. J. Camelid Sci., 5, 1-24.

Wheeler, J.C., Russel, A.J.F., Redden, H., 1995. Llamas and alpacas: Pre-conquest breeds and post-conquest hybrids. J. Arch. Sci., 22, 833-840.