Asian Journal of Andrology (2016) 18, 844–850

www.asiaandro.com; www.ajandrology.com

Open Access

INVITED REVIEW

Sperm Biology

Importance of sperm morphology during sperm transport and fertilization in mammals

Francisco A García-Vázquez[1,2], Joaquín Gadea[1,2], Carmen Matás[1,2], William V Holt[3]

After natural or artificial insemination, the spermatozoon starts a journey from the site of deposition to the place of fertilization. However, only a small subset of the spermatozoa deposited achieves their goal: to reach and fertilize the egg. Factors involved in controlling sperm transport and fertilization include the female reproductive tract environment, cell-cell interactions, gene expression, and phenotypic sperm traits. Some of the significant determinants of fertilization are known (i.e., motility or DNA status), but many sperm traits are still indecipherable. One example is the influence of sperm dimensions and shape upon transport within the female genital tract towards the oocyte. Biophysical associations between sperm size and motility may influence the progression of spermatozoa through the female reproductive tract, but uncertainties remain concerning how sperm morphology influences the fertilization process, and whether only the sperm dimensions *per se* are involved. Moreover, such explanations do not allow the possibility that the female tract is capable of distinguishing fertile spermatozoa on the basis of their morphology, as seems to be the case with biochemical, molecular, and genetic properties. This review focuses on the influence of sperm size and shape in evolution and their putative role in sperm transport and selection within the uterus and the ability to fertilize the oocyte.
Asian Journal of Andrology (2016) **18**, 844–850; doi: 10.4103/1008-682X.186880; published online: 13 September 2016

Keywords: CASA-Morph; female reproductive tract; flagellum; morphometry; sperm competition; sperm head; sperm selection; sperm size

INTRODUCTION

A huge number of spermatozoa are deposited in the female genital tract at ejaculation, but little is known about the special characteristics which enable a particular spermatozoon to reach the oocyte and fertilize it in preference to the other millions around it. Within the female genital tract, spermatozoa have to negotiate different physical barriers and undergo complex interactions. In some species (i.e., humans, sheep), the cervix presents an important obstacle and is the first "filter" for abnormal spermatozoa.[1] Once in the uterus, spermatozoa are in contact with uterine fluid, which, in species such as the mouse, induces deleterious effects on spermatozoa unless they are provided with protein SVS2 present in seminal plasma.[2] Moreover, within the uterus, spermatozoa make contact with different cell types (cell-cell interaction), among them polymorphonuclear leukocytes (PMNs), that are present in the lumen of the uterus after insemination. It is not clear yet whether the PMNs are able to distinguish between abnormal and normal spermatozoa, although damaged, capacitated, and moribund spermatozoa seem to be eliminated by phagocytosis.[3–5] Once in the oviduct, the environment is more suitable for promoting sperm viability. In this respect, leukocytes in this anatomical region are sparse or even absent[6] and the interaction with oviductal epithelial cells and oviductal fluid modulates sperm function.[7,8] Moreover, the arrival of spermatozoa in the oviduct modulates local gene expression, thus preparing the oviduct for an adequate environment for gamete interactions and early embryo development.[9] Surprisingly, the "sex of the sperm cell" appears to modify the oviductal transcriptome in a sex-specific manner.[10] Once in the oviduct, the epithelial cells appear to exert rigorous selection, being able to bind spermatozoa with particular characteristics such as normal chromatin,[8] lack of capacitation, or morphological "normality"[11,12] among others. The failure of abnormal spermatozoa to reach the site of fertilization has also been demonstrated in various species.[13,14]

One hypothesis is that females can select which spermatozoa reach and fertilize the oocyte;[15,16] this introduces the concept of the "sperm passport." Just as each individual person has some characteristics that make him different from others, i.e., biometric identifiers which are physiological characteristics related commonly to the shape of the body, each spermatozoon could have its own biometric identifier, making it able (or not) to progress along the female tract and fertilize. Conversely, females could use such molecular mechanisms to recognize and obtain the information from an individual spermatozoon.[15]

Moreover, it is known that an ejaculate is composed of different subpopulations of spermatozoa.[5] The subpopulations are characterized by differences in motility, DNA fragmentation status, morphology or shape and size, sensitivity to signaling molecules, and many other properties. Motility is very important; when females are inseminated with spermatozoa exhibiting different levels of motility, those spermatozoa with poor motility are found in the backflow after only

[1]Department of Physiology, Faculty of Veterinary Science, International Excellence Campus for Higher Education and Research "Campus Mare Nostrum", University of Murcia, Murcia 30100, Spain; [2]Institute for Biomedical Research of Murcia (IMIB-Arrixaca), Murcia, Spain; [3]Department of Human Metabolism, Academic Unit of Reproductive and Developmental Medicine, Sheffield S10 2SF, UK.
Correspondence: Dr. FA García-Vázquez (fagarcia@um.es)

15 min.[17] This indicates that an initial sperm selection process within the genital tract is biased in favor of highly motile spermatozoa. With regard to the sperm chromatin, other authors have demonstrated the superior ability of spermatozoa with stable chromatin to reach the fertilization site[18] and bind to the zona pellucida.[19]

What makes spermatozoa successful in reaching the site of fertilization and fertilizing the egg depends on some of the traits mentioned above (i.e., good motility, adequate morphology, and normal DNA status). Sperm heterogeneity in an ejaculate may have functional relevance, ensuring a greater potential to fertilize after being deposited in the female genital tract. The aim of the present review is focused on the influence of sperm size and shape in evolution and their putative role in sperm transport and selection within the uterus and the ability to fertilize the oocyte.

DIFFERENCES IN MAMMALIAN SPERM MORPHOLOGY

As explained above, the mechanisms that determine how spermatozoa are transported in the female genital tract are still controversial, as is the significance of variations in mammalian sperm morphology. Why, for example, do the spermatozoa vary in shape and size between species, and even within an ejaculate? Even within a single taxonomic group such as the rodents, sperm heads have evolved a remarkable spectrum of shapes. While mouse, rat, and hamster spermatozoa are hook-shaped, the sperm heads of some Australian rodents of the genus *Pseudomys* have also developed two auxiliary hooks[20,21] that are not extensions of the acrosome. In contrast, the hystricomorph rodents, including the guinea pig,[22] degu,[23] and *Chinchilla*,[24] have evolved exceptionally large acrosomes, a feature also found in some insectivores such as the white-tailed shrew.[25]

Spermatozoa are subjected to postmating sexual selection within the female tract, and it has been suggested that the dimensions of different sperm components are responsible for the continuous adjustments of male fertility, which eventually produce these dramatic phenotypic differences.[16] Species with high levels of sperm competition are believed to produce longer spermatozoa than others because, from a purely biophysical perspective, the positive correlation between flagellar length and swimming speed[26] should enable the faster spermatozoa to reach the uterotubal junction (UTJ) before their rivals. However, major studies in mammals have failed to support this simple hypothesis,[27,28] and this relationship also oversimplifies what happens when spermatozoa are swimming within the viscous environment of the uterus and oviducts[29] and their flagellar activity is modified.[30,31] When spermatozoa are swimming within the female reproductive tract, they are subjected to more complex influences than, for example, fish spermatozoa in freshwater or seawater.

Mammalian spermatozoa have a tendency to interact with adjacent cells and, therefore, to swim along epithelial surfaces.[32] A recent study of this phenomenon indicated that human spermatozoa exhibit "slither," or two-dimensional, swimming within the female reproductive tract, which involves remaining in very close contact to the epithelial cells.[33] This mode of swimming allows human spermatozoa to make faster progress than bull spermatozoa under similar conditions because the latter remain further away from the epithelial cell layer. In addition to the physical differences imposed by flagellar length and structure, female reproductive tract anatomy also modulates the progress of spermatozoa. In some species, such as sheep, pigs, and cows, spermatozoa adhere firmly to epithelial cells of the oviductal isthmus, where they form a sperm reservoir,[32,34,35] while in species such as the musk shrew[36] and the Australian dasyurid marsupial, *Sminthopsis*,[37] the spermatozoa reside for a period in epithelial crypts and, therefore, do not have to swim toward the oocyte immediately after mating.

Total sperm length varies from 28.30 to 258.33 μm across more than 200 mammalian species analyzed.[28,38,39] Considering only nondomestic species (**Table 1** and **Supplementary Table 1**), there is considerable variation in sperm dimensions. For example, total sperm length ranges from 28.30 to 189.40 μm in 193 of the species measured, which represents an increment of more than 500% between minimal and maximal value. Such data have to be viewed in the context of evolution and analyzed with specialized statistical techniques that incorporate phylogenetic information.[40] In the case of domestic animals (**Table 2** and **Supplementary Table 2**), this variation between species is less pronounced (i.e., total sperm length ranges from 47.21 to 114.07 μm), although only 22 domestic species were analyzed. When more common domestic farm animals were considered (**Table 3**), the sperm dimensions were quite similar across species. Although the effects of sperm competition on sperm size is still not resolved,[41] the reduction in variation of dimension mentioned above might be a consequence of the reduction in between-male competition in domestic animals, as a result of selection of genetically high-value individuals. Little is known about how the level of sperm competition might affect some sperm traits,[42] although a significant study of mole rat spermatozoa, where because of the unusual social system there is little or no sperm competition, revealed a massive degree of within-ejaculate structural diversity, low incidence of motile spermatozoa, and slow swimming speed.[43] In this sense, sperm characteristics seem to depend on social environment, as occurs in horses, where the presence of other males and mares influences ejaculate sperm concentration and motion parameters.[44]

When females are artificially inseminated with spermatozoa from two or more males (heterospermic insemination dosages), spermatozoa from specific males are consistently favored in their ability

Table 1: Sperm dimension ranges in up to 193 nondomestic mammalian species (data collected from Tourmente *et al*.[39])

Sperm traits	*Number of species measured*	*Minimum*	*Maximum*	*Increment (%)#*
Head width (μm)	65	2.00	12.20	510.00
Head length (μm)	164	3.00	15.00	400.00
Midpiece length (μm)	164	3.30	67.00	1930.30
Principal piece length (μm)	164	15.60	125.00	7012.82
Total flagellum length (μm)	164	22.00	177.00	704.54
Total sperm length (μm)	193	28.30	189.40	569.25

#Increment (%) parameter indicates the difference between the lowest to the highest value among species

Table 2: Sperm dimension ranges in up to 22 domestic mammalian species (data collected from Tourmente *et al*.[39])

Sperm traits	*Number of species measured*	*Minimum*	*Maximum*	*Increment (%)#*
Head width (μm)	12	3.30	6.60	100
Head length (μm)	20	4.50	10.87	141.55
Midpiece length (μm)	20	5.30	15.45	191.51
Principal piece length (μm)	20	34.23	92.10	169.06
Total flagellum length (μm)	20	41.57	103.20	148.25
Total sperm length (μm)	22	47.21	114.07	141.62

#Increment (%) parameter indicates the difference between the lowest to the highest value among species

Table 3: Sperm dimensions of some domestic mammalian species (data collected from Tourmente *et al.*[39])

Sperm traits	*Equus caballus Stallion*	*Sus scrofa Boar*	*Bos taurus Bull*	*Ovis aries Ram*	*Capra hircus Goat*	*Increment (%)#*
Head width (μm)	3.90**	4.41*	4.30	4.30	4.25**	13.07
Head length (μm)	7.00	9.08*	6.77	8.20	8.27	34.12
Midpiece length (μm)	9.80	10.00	9.38	14.00	11.38	49.25
Principal piece length (μm)	43.80	36.10	36.93	42.50	39.75	21.33
Total flagellar length (μm)	53.60	46.57*	46.76	56.50	51.13	21.32
Total sperm length (μm)	60.60	55.65*	53.53	64.70	59.39	20.86

#Increment (%) parameter indicates the difference between the lowest to the highest value among species.*Garcia-Vazquez *et al.*;[56] **Cummins and Woodall[38]

to fertilize oocytes and produce offspring.[45–48] This indicates either a significant female selection of spermatozoa or sperm traits *per se*, and in both cases, morphological characteristics could be involved. The spermatozoa within an ejaculate are typically recognizable as belonging to different subpopulations whereby one cell type can have a higher fertilization potential.[49] For example, boar sperm heads from an ejaculate can be morphometrically divided into three subpopulations: large, small-elongated, and small round sperm populations[50] or rectangular-shaped, sharply tapering, or slightly tapering sperm heads.[51] Buck (goat), bull, and ram spermatozoa can be similarly recognized as belonging to different subpopulations.[50] The biological relevance of these subpopulations remains unclear, and further research is necessary to clarify what some of the variation really means. In human clinical medicine, the concept of the "normal" spermatozoon is widely used and the latest version of the World Health Organization guidelines on semen analysis[52] indicates that only about 4% of spermatozoa within a fertile human ejaculate conform to the morphological definition of a normal spermatozoon. The definition was derived by examining the morphology of spermatozoa that reached the vicinity of the oocyte after insemination. Viewed from the perspective of comparative biology, this is rather similar to the situation with the naked mole rat cited above,[42,43] where the absence of sperm competition has relaxed the pressure to produce a high proportion of uniformly competent spermatozoa during spermatogenesis. Anthropological evidence confirms that as a species, humans have evolved in a context where sperm competition is not an important influence.[53] Nevertheless, this observation also highlights that the human female reproductive tract must be capable of selectively preventing abnormal spermatozoa from migrating through the reproductive tract and accessing the oocytes.

SPERM SELECTION WITHIN THE FEMALE GENITAL TRACT RELATED TO MORPHOLOGY

As mentioned above, the small number of spermatozoa reaching the oviduct and deposited in the sperm reservoir is the result of strong selection during sperm transport in the female genital tract. Only morphologically normal spermatozoa, uncapacitated and with intact DNA are capable of binding to oviductal epithelia.[8] However, the number of spermatozoa with these characteristics present in an ejaculate is much higher than those that reach the reservoir. Therefore, the female genital somehow carries out other more complex selection so that it will choose the spermatozoon that fertilizes the egg, i.e., "cryptic female selection"[54,55] (**Figure 1**).

Since the environment in the cervix and uterus is hostile to spermatozoa, they have to swim actively to move forward along the upper sections of the female tract. The first types of excluded spermatozoa are likely to be those with midpiece or tail defects that impair motility. Garcia-Vazquez *et al.*[56] have shown that spermatozoa analyzed in the backflow were small (head and flagellum), with different head shapes compared with spermatozoa observed in the dose before insemination. The site of deposition (cervix *vs* uterus) also influences sperm selection, head morphometry and tail size both being smaller in the backflow after cervical insemination.

In the uterus, the seminal plasma (SP) has an important role in affecting sperm motility and the maintenance of viability. Spermatozoa that reach the uterus may not be able to swim through the UTJ unless they possess certain cell surface proteins derived from the SP.[57,58] In addition, the uterus induces sperm cell death and some proteins from SP protect sperm from uterine attack.[2] SP also provides energy substrates that drive oxidative phosphorylation, stabilize the sperm plasma membrane, and prevent uterine spermatozoa from undergoing premature capacitation and the acrosome reaction.[59]

The energy for supporting the key functions of the spermatozoa, including motility, is provided by adenosine triphosphate (ATP). The ATP is formed through two metabolic pathways: glycolysis and oxidative phosphorylation (OXPHOS). Glycolysis occurs in the principal piece of the flagellum and the OXPHOS takes place in the mitochondria, which are tightly packed in the sperm midpiece. The amount of ATP produced by OXPHOS is much higher than that produced by glycolysis, and for this reason, this pathway is considered as the main source of ATP production for sperm motility.[60] On the basis of these characteristics, one might think that spermatozoa with a longer midpiece will be the faster; however, there is a wide range of variation of this relationship among mammalian species. For example, Malo *et al.*[61] observed that spermatozoa with longer midpieces swam more slowly and spermatozoa with elongated heads, and those in which the relative length of the rest of the flagellum is longer, swam faster. However, Anderson *et al.*[62] showed, in a study using 494 specimens, that the volume of the midpiece (but not the length) was higher in those mammals where females have a multiple-partner mating system. Presumably, a larger volume midpiece has higher densities of mitochondria, which could provide an increase in sperm motility and an advantage at the level of sperm competition.

Gomendio and Roldan[63] found that spermatozoa with elongated heads swam faster and that the effect of head shape upon sperm hydrodynamics was considerable. However, Mossman *et al.*[64] showed that the mean flagellar length and the mean total sperm length were not associated with the sperm swimming speed, measured by computer-aided sperm analysis. Nevertheless, boar spermatozoa with both short and long flagella were able to reach and colonize the oviductal sperm reservoir.[65] Hence, it seems that the factors determining the sperm arrival to the place of fertilization are sperm swimming velocity, the shape of the head, and the forces generated by the relative size of the rest of the flagellum.

On the other hand, studies of ram spermatozoa suggest that spermatozoa with large and long heads are more fertile than those with smaller heads, and that this morphometric parameter could be

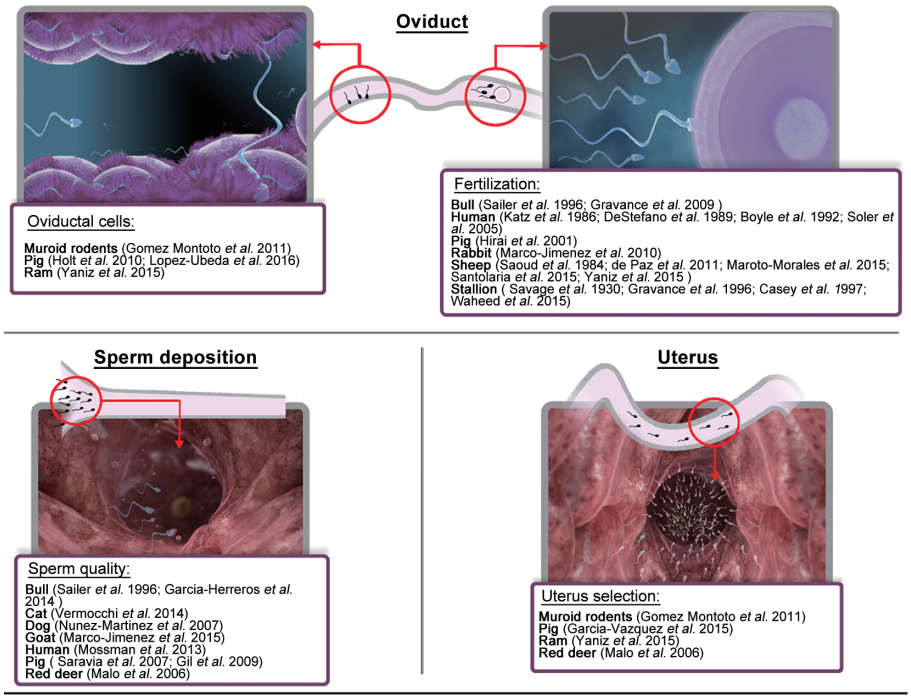

Figure 1: References related to sperm morphology in different situations within the female genital tract: sperm quality before or during deposition, sperm selection during their travel within the uterus, influence of sperm morphology during the interaction with oviductal epithelial cells and fertilization.

an indicator of fertility.[66] When spermatozoa reach the oviduct, they attach to the epithelium (**Figure 1**); those that do not are unable to survive.[67] Gomez Montoto *et al*.[40] proposed that an increase in sperm head size may facilitate interactions and attachment with oviductal epithelial cells, and that it may also obstruct the attachment of rival spermatozoa. However, despite all of the above, it seems that sperm selection is based on more complex criteria involving male genotype[68] and/or "cryptic female choice."[15,54]

RELATIONSHIPS BETWEEN SPERM MORPHOLOGY AND FERTILITY AND OTHER SPERM QUALITY PARAMETERS

Sperm morphology and fertility

The common use of spermatozoa in artificial insemination protocols in a great number of species constantly reinvigorates interest in the identification of subfertile or infertile males. That is why the relationships between sperm morphometry and fertility have been the objective of a large number of studies for nearly a century[69] (**Figure 1**). However, the results reported until now have not been totally conclusive. Different factors affect the morphometric sperm measurements; some are related to sample variability with respect to season,[70,71] age,[72] breeds or subspecies,[73–76] and sexual maturity.[77] Differences also arise through the measurement procedures themselves, including the staining procedure,[78] microscopic evaluation technique,[79] wet versus stained samples,[80] sperm preparation,[81] and statistical methodology (mean value, variation coefficient, and subpopulation analysis)[82,83] among others. Differences in the methodology of the studies and the large number of factors that affect the relationships could be behind the ambiguous results reported in the scientific literature. We will try to analyze the main results reported in some species.

The reported studies have tried to measure the sperm head dimensions, flagellar length,[64] midpiece length,[73] or the nuclear shape[84,85] and to relate them to fertility. Differences in sperm head length have been directly related to conception rates in some species. For example, an increase in the coefficient of variation of the sperm head length in bulls and stallions has been related to a reduction of fertility.[69,83] However, no correlation was found in bulls when the deviation of morphometric head parameters was analyzed with respect to fertility ratings.[86] Other studies have included the relationship of morphometry with sperm cryopreservation. In this sense, the post-thaw sperm survival and fertility rates for bulls and boars were correlated with prefreeze measurements of width and the change in width/length after cryopreservation.[51,87]

For humans, higher sperm head width and lower length/width ratios were detected in spermatozoa from fertile than infertile men.[80] However, other authors did not find morphometric measurements useful in predicting fertility.[88] In an interesting study with American soldiers, Vietnam veterans showed longer mean sperm head major axis length and head circumference than coetaneous veteran soldiers in other areas with similar fertility.[89] Later, in a study of greater size, the sperm length/width ratio was confirmed as an important parameter in relation to impaired fertility.[90] An inverse relationship between sperm head area and perimeter with fertility after human intrauterine insemination (IUI) and intracytoplasmic sperm injection (ICSI) has also been reported.[91]

Another common domestic animal studied has been the horse. In stallions, higher values for sperm head length, area, and perimeter are found in subfertile rather than in fertile animals.[92,93] The sperm head area and perimeter were smaller in stallions with high fertility (69%–79%) than in those of low fertility (50%–59%).[71] Although the number of reports is still limited, the data suggest that differences in the dimensions of sperm heads may exist between fertile and subfertile stallions.

For the pig breeding industry, different approaches have been developed to find fertility markers, such as the use of sperm protein profiles.[94] The finding of a correlation between sperm morphometry and fertilization might be very useful because of the huge number of sperm doses used every day for artificial insemination. When the sperm head morphometry from boars with high fertility (nonreturn rate >86%) versus lower fertility (<86%) was compared,[95] the authors found that high fertility was related to lower values in sperm head area and length, and higher values for width and ratio width/length. Similarly, they compared sperm head dimensions between groups with litter sizes >10 and <10 live-born piglets, obtaining the opposite results.[95] They found higher area and length of sperm heads, and lower width and width/length ratios, in the group of higher litter size (>10). The authors did present an explanation for their results[95] and a comparison with the results in other species (i.e., horses[92]) was not appropriate because of the differences between evaluations of fertility parameters among species.

For rabbits, sperm head morphometry parameters are heritable[96] and males with smaller sperm head size show lower fertility (45.0% *vs* 77.9%).[97] Nevertheless, the sperm head morphometric parameters assayed showed low potential to predict fertility and litter size when the ejaculates fulfilled the minimum requirements commonly used in artificial insemination (motility and percentage of abnormal spermatozoa).[98]

The sheep has been one of the species studied for relating fertility and sperm morphometry. According to de Paz *et al*.,[79] the relationship between ram sperm head morphometry and fertility depends on the methodology of evaluation, with the best results being obtained when a system based on light microscopy with a digital camera and a conventional image analysis is used. However, ram sperm midpiece length was not related to fertility.[73] An interesting approach has recently been reported in the study of ram sperm morphometric subpopulations and their relationship with fertility.[49,66,82] While no relationships were found between male fertility rates and average values of sperm head dimensions, differences in fertility rates between rams were strongly associated with the proportion of spermatozoa in an ejaculate with short and elongated heads.[82] The distribution of subpopulations between rams of high and low field fertility was different, with higher percentages of spermatozoa exhibiting fast and linear movements, and those with large and long nuclei in the high fertility group.[66] However, the importance of morphometric values is relative for predicting fertility, because when the morphometric values were evaluated, together with viability, DNA fragmentation rate, and motility values in a logistic regression model for ovine fertility rate, only the viability and VCL needed to be included in the model.[49]

It is not currently possible to distinguish the real cause of the differences observed between species. The results relating morphology and fertility in literature are controversial and sometimes contrary between species, as pointed out throughout the review. For example, for pigs, small head area is associated with higher fertility[95] whereas in rabbits small sperm heads are linked with lower fertility.[97] Therefore, other mechanisms may be taken into account relative to intraspecies reproductive factors such as site of sperm deposition, length of the uterus, or number of sperm deposited, among others. For example, in some species (i.e., human and rams), cervical mucus acts as one of the main barriers, so shape might be essential for sperm in order to make their way through the mesh-like structure of cervical mucus, and the hydrodynamic design of the sperm head would influence the sperm swimming within the female genital tract,[63] so it may favor spermatozoa whose shape is best adapted to swim under these constraints. Hence, maybe we cannot directly compare the results obtained related to the morphology between different species. In fact, some authors[50] have reported different subpopulations in four species of domestic animals (cattle, sheep, goat, and pigs), with the size category classification being different between the four species.

Sperm morphology related with other sperm qualities

From a comparative point of view, different authors have studied the possible relationships between sperm dimensions and body weight or other parameters such as genome mass, chromosome number, or duration of estrus in different species.[27,38,99] Furthermore, some studies have evaluated possible relationships between sperm morphometry and other seminal parameters (i.e., sperm concentration or motility) (**Figure 1**).[64,100] Relationships between motion parameters measured by CASA-Mot (VCL, ALH, STR, and LIN) and sperm morphometry have been reported in pigs[100] and deer.[61] In the case of pigs, boar sperm head and intermediate piece morphometry influence their motility characteristics;[100] for example, larger mean values of sperm head area are associated with larger mean values of ALH, a parameter which is related with sperm hyperactivation. This finding could be interesting in fertility programs or as a model to evaluate the capacitation process.

One objective was to evaluate any possible relationship between sperm head morphometry and the chromatin structure. Some authors have studied this relationship in the domestic bull,[86] pigs,[76] and carnivores.[101,102] A consistent relationship between the standard deviation of morphometric head parameters and chromatin structure measured by SCSA has been detected in bulls.[86] In contrast, Saravia *et al*.[76] studied porcine spermatozoa and did not find any consistent relationship between morphometry and SCSA outcomes. In the same way, no differences were found between head morphometry parameters and DNA fragmentation index in feline epididymal spermatozoa.[101] In dogs, a significant relationship has been reported between sperm head length (inverse) and sperm head width (direct) with the percentage of DNA fragmentation measured by SCSA.[102] The shape of the sperm nuclei appeared to be more informative about chromatin structure than morphometry.[84,85]

Sperm viability has also been related with morphometry. In goats, dead spermatozoa are smaller in head length, width, area, and perimeter than live ones after freezing-thawing. This fact probably is associated with the loss of sperm membrane function.[103] Similarly, bull spermatozoa that regarded as dead after cryopreservation show smaller dimensions than those that survived, suggesting that sperm morphometry could be a valuable tool for detecting changes associated with sperm membrane integrity in these conditions.[104]

Finally, some authors have evaluated sperm morphometry after freezing in different species such as dog,[105] goats,[103,106,107] bulls,[108] and boars.[51] These studies have tried to provide a forecast of the freezing ability, resistance to the cryopreservation process and the value in predicting post-thaw fertility.

CONCLUSIONS

It would seem to be obvious that sperm selection within the female genital tract depends on different factors, and that sperm morphology could be one of them. Nonetheless, it is far from clear how to understand the mechanisms that relate sperm trait morphometry and their selection in the quest for female gamete encounter. New knowledge about sperm size implications in fertility could offer to the reproductive biotechnology industry new tools for semen evaluation, thus turning morphometry into

a reliable and predictive test for potential fertility. This would enable the selection of those ejaculates with determined morphometry properties measured by CASA-Morph as happens routinely with other sperm traits, such as motility evaluation. However, this is not possible at present because much remains to be discovered about the meaning of sperm morphometry.

AUTHOR CONTRIBUTIONS

FAGV conceived and designed the manuscript; CM, JG, WVH, and FAGV drafted the manuscript; all authors read and approved the final version of the manuscript.

COMPETING INTERESTS

The authors declared that they have no competing interests.

ACKNOWLEDGMENTS

The results cited by the authors were funded by the Spanish Ministry of Economy and Competitiveness (MINECO) and the European Regional Development Fund (FEDER) (Grant AGL2015-66341-R) and the Seneca Foundation of Murcia (20040/GERM/16).

Supplementary information is linked to the online version of the paper on the *Asian Journal of Andrology* website.

REFERENCES

1 Mattner PE. Formation and retention of the spermatozoan reservoir in the cervix of the ruminant. *Nature* 1966; 212: 1479–80.
2 Kawano N, Araki N, Yoshida K, Hibino T, Ohnami N, *et al*. Seminal vesicle protein SVS2 is required for sperm survival in the uterus. *Proc Natl Acad Sci U S A* 2014; 111: 4145–50.
3 Schuberth HJ, Taylor U, Zerbe H, Waberski D, Hunter R, *et al*. Immunological responses to semen in the female genital tract. *Theriogenology* 2008; 70: 1174–81.
4 Taylor U, Rath D, Zerbe H, Schuberth HJ. Interaction of intact porcine spermatozoa with epithelial cells and neutrophilic granulocytes during uterine passage. *Reprod Domest Anim* 2008; 43: 166–75.
5 Holt WV, Fazeli A. Sperm selection in the female mammalian reproductive tract. Focus on the oviduct: hypotheses, mechanisms, and new opportunities. *Theriogenology* 2016; 85: 105–12.
6 Rodriguez-Martinez H, Nicander L, Viring S, Einarsson S, Larsson K. Ultrastructure of the uterotubal junction in preovulatory pigs. *Anat Histol Embryol* 1990; 19: 16–36.
7 Coy P, Aviles M. What controls polyspermy in mammals, the oviduct or the oocyte? *Biol Rev Camb Philos Soc* 2010; 85: 593–605.
8 Lopez-Ubeda R, Garcia-Vazquez FA, Gadea J, Matas C. Oviductal epithelial cells selected boar sperm according to their functional characteristics *Asian J Androl* 2016; Doi: 10.4103/1008-682X.173936; [Epub ahead of print].
9 Lopez-Ubeda R, Garcia-Vazquez FA, Romar R, Gadea J, Munoz M, *et al*. Oviductal transcriptome is modified after insemination during spontaneous ovulation in the sow. *PLoS One* 2015; 10: e0130128.
10 Alminana C, Caballero I, Heath PR, Maleki-Dizaji S, Parrilla I, *et al*. The battle of the sexes starts in the oviduct: modulation of oviductal transcriptome by X and Y-bearing spermatozoa. *BMC Genomics* 2014; 15: 293.
11 Petrunkina AM, Gehlhaar R, Drommer W, Waberski D, Topfer-Petersen E. Selective sperm binding to pig oviductal epithelium *in vitro*. *Reproduction* 2001; 121: 889–96.
12 Luno V, Lopez-Ubeda R, Garcia-Vazquez FA, Gil L, Matas C. Boar sperm tyrosine phosphorylation patterns in the presence of oviductal epithelial cells: *in vitro*, *ex vivo*, and *in vivo* models. *Reproduction* 2013; 146: 315–24.
13 Garcia-Vazquez FA, Hernandez-Caravaca I, Matas C, Soriano-Ubeda C, Abril-Sanchez S, *et al*. Morphological study of boar sperm during their passage through the female genital tract. *J Reprod Dev* 2015; 61: 407–13.
14 Mortimer D, Leslie EE, Kelly RW, Templeton AA. Morphological selection of human spermatozoa *in vivo* and *in vitro*. *J Reprod Fertil* 1982; 64: 391–9.
15 Holt WV, Fazeli A. Do sperm possess a molecular passport? Mechanistic insights into sperm selection in the female reproductive tract. *Mol Hum Reprod* 2015; 21: 491–501.
16 Fitzpatrick JL, Lupold S. Sexual selection and the evolution of sperm quality. *Mol Hum Reprod* 2014; 20: 1180–9.
17 Hernandez-Caravaca I, Soriano-Ubeda C, Matas C, Izquierdo-Rico MJ, Garcia-Vazquez FA. Boar sperm with defective motility are discriminated in the backflow moments after insemination. *Theriogenology* 2015; 83: 655–61.
18 Ardon F, Helms D, Sahin E, Bollwein H, Topfer-Petersen E, *et al*. Chromatin-unstable boar spermatozoa have little chance of reaching oocytes *in vivo*. *Reproduction* 2008; 135: 461–70.
19 Tsakmakidis IA, Lymberopoulos AG, Khalifa TA. Selectivity of porcine zona pellucida to bind spermatozoa with normal chromatin structure. *Andrologia* 2011; 43: 358–60.
20 Breed WG. Variation in sperm morphology in the Australian rodent genus, *Pseudomys* (*Muridae*). *Cell Tissue Res* 1983; 229: 611–25.
21 Breed WG. Variation in sperm form in rats and mice (subfamily *Murinae*). Proceeding of 9th International Symposium on Spermatology. Cape Town, South Africa; 2002. p. 269–74.
22 Holt WV. Development and maturation of the mammalian acrosome. A cytochemical study using phosphotungstic acid staining. *J Ultrastruct Res* 1979; 68: 58–71.
23 Berrios M, Flechon JE, Barros C. Ultrastructure of *Octodon degus* spermatozoon with special reference to the acrosome. *Am J Anat* 1978; 151: 39–53.
24 Fawcett DW, Anderson WA, Phillips DM. Morphogenetic factors influencing the shape of the sperm head. *Dev Biol* 1971; 26: 220–51.
25 Bedford JM, Phillips DM, Mover-Lev H. Novel sperm crypts and behavior of gametes in the fallopian tube of the white-toothed shrew, *Crocidura russula* monacha. *J Exp Zool* 1997; 277: 262–73.
26 Dresdner RD, Katz DF. Relationships of mammalian sperm motility and morphology to hydrodynamic aspects of cell function. *Biol Reprod* 1981; 25: 920–30.
27 Gage MJ, Freckleton RP. Relative testis size and sperm morphometry across mammals: no evidence for an association between sperm competition and sperm length. *Proc R Soc Lond B Biol Sci* 2003; 270: 625.
28 Lupold S, Fitzpatrick JL. Sperm number trumps sperm size in mammalian ejaculate evolution. *Proc Biol Sci* 2015; 282: pii: 20152122.
29 Smith DJ, Gaffney EA, Gadelha H, Kapur N, Kirkman-Brown JC. Bend propagation in the flagella of migrating human sperm, and its modulation by viscosity. *Cell Motil Cytoskeleton* 2009; 66: 220–36.
30 Babcock DF, Wandernoth PM, Wennemuth G. Episodic rolling and transient attachments create diversity in sperm swimming behavior. *BMC Biol* 2014; 12: 67.
31 Hyakutake T, Suzuki H, Yamamoto S. Effect of non-Newtonian fluid properties on bovine sperm motility. *J Biomech* 2015; 48: 2941–7.
32 Suarez SS. Mammalian sperm interactions with the female reproductive tract. *Cell Tissue Res* 2016; 363: 185–94.
33 Nosrati R, Driouchi A, Yip CM, Sinton D. Two-dimensional slither swimming of sperm within a micrometre of a surface. *Nat Commun* 2015; 6: 8703.
34 Hunter RH. Components of oviduct physiology in eutherian mammals. *Biol Rev Camb Philos Soc* 2012; 87: 244–55.
35 Croxatto HB. Physiology of gamete and embryo transport through the fallopian tube. *Reprod Biomed Online* 2002; 4: 160–9.
36 Bedford JM, Cooper GW, Phillips DM, Dryden GL. Distinctive features of the gametes and reproductive tracts of the Asian musk shrew, *Suncus murinus*. *Biol Reprod* 1994; 50: 820–34.
37 Bedford JM, Breed WG. Regulated storage and subsequent transformation of spermatozoa in the fallopian tubes of an Australian marsupial, *Sminthopsis crassicaudata*. *Biol Reprod* 1994; 50: 845–54.
38 Cummins JM, Woodall PF. On mammalian sperm dimensions. *J Reprod Fertil* 1985; 75: 153–75.
39 Tourmente M, Gomendio M, Roldan ER. Sperm competition and the evolution of sperm design in mammals. *BMC Evol Biol* 2011; 11: 12.
40 Gomez Montoto L, Varea Sanchez M, Tourmente M, Martin-Coello J, Luque-Larena JJ, *et al*. Sperm competition differentially affects swimming velocity and size of spermatozoa from closely related muroid rodents: head first. *Reproduction* 2011; 142: 819–30.
41 Snook RR. Sperm in competition: not playing by the numbers. *Trends Ecol Evol* 2005; 20: 46–53.
42 van der Horst G, Maree L. Sperm form and function in the absence of sperm competition. *Mol Reprod Dev* 2014; 81: 204–16.
43 van der Horst G, Maree L, Kotze SH, O'Riain MJ. Sperm structure and motility in the eusocial naked mole-rat, *Heterocephalus glaber*: a case of degenerative orthogenesis in the absence of sperm competition? *BMC Evol Biol* 2011; 11: 351.
44 Burger D, Dolivo G, Wedekind C. Ejaculate characteristics depend on social environment in the horse (*Equus caballus*). *PLoS One* 2015; 10: e0143185.
45 Kasimanickam R, Nebel RL, Peeler ID, Silvia WL, Wolf KT, *et al*. Breed differences in competitive indices of Holstein and Jersey bulls and their association with sperm DNA fragmentation index and plasma membrane integrity. *Theriogenology* 2006; 66: 1307–15.
46 Ferreira CE, Savio DB, Guarise AC, Flach MJ, Gastal GD, *et al*. Contribution of boars to reproductive performance and paternity after homospermic and heterospermic artificial insemination. *Reprod Fertil Dev* 2015; 27: 1012–9.
47 Dziuk PJ. Factors that influence the proportion of offspring sired by a male following heterospermic insemination. *Anim Reprod Sci* 1996; 43: 65–88.
48 Stahlberg R, Harlizius B, Weitze KF, Waberski D. Identification of embryo paternity using polymorphic DNA markers to assess fertilizing capacity of spermatozoa after heterospermic insemination in boars. *Theriogenology* 2000; 53: 1365–73.
49 Santolaria P, Vicente-Fiel S, Palacin I, Fantova E, Blasco ME, *et al*. Predictive capacity of sperm quality parameters and sperm subpopulations on field fertility after artificial insemination in sheep. *Anim Reprod Sci* 2015; 163: 82–8.
50 Vicente-Fiel S, Palacin I, Santolaria P, Yaniz JL. A comparative study of

sperm morphometric subpopulations in cattle, goat, sheep and pigs using a computer-assisted fluorescence method (CASMA-F). *Anim Reprod Sci* 2013; 139: 182–9.
51 Thurston LM, Watson PF, Mileham AJ, Holt WV. Morphologically distinct sperm subpopulations defined by Fourier shape descriptors in fresh ejaculates correlate with variation in boar semen quality following cryopreservation. *J Androl* 2001; 22: 382–94.
52 WHO. WHO Laboratory Manual for the Examination and Processing of Human Semen. 5th ed. Geneva: World Health Organization; 2010. p. 286.
53 Martin RD. The evolution of human reproduction: a primatological perspective. *Am J Phys Anthropol* 2007; Suppl 45: 59–84.
54 Birkhead TR. Cryptic female choice: criteria for establishing female sperm choice. *Evolution* 1998; 52: 1212.
55 Telford SR, Jennions MD. Establishing cryptic female choice in animals. *Trends Ecol Evol* 1998; 13: 216–8.
56 Garcia-Vazquez FA, Hernandez-Caravaca I, Yanez-Quintana W, Matas C, Soriano-Ubeda C, *et al*. Morphometry of boar sperm head and flagellum in semen backflow after insemination. *Theriogenology* 2015; 84: 566–74.
57 Suarez SS. Interactions of spermatozoa with the female reproductive tract: inspiration for assisted reproduction. *Reprod Fertil Dev* 2007; 19: 103–10.
58 Fujihara Y, Tokuhiro K, Muro Y, Kondoh G, Araki Y, *et al*. Expression of TEX101, regulated by ACE, is essential for the production of fertile mouse spermatozoa. *Proc Natl Acad Sci U S A* 2013; 110: 8111–6.
59 Poiani A. Complexity of seminal fluid: a review. *Behav Ecol Sociobiol* 2006; 60: 289–310.
60 Tourmente M, Villar-Moya P, Rial E, Roldan ER. Differences in ATP generation via glycolysis and oxidative phosphorylation and relationships with sperm motility in mouse species. *J Biol Chem* 2015; 290: 20613–26.
61 Malo AF, Gomendio M, Garde J, Lang-Lenton B, Soler AJ, *et al*. Sperm design and sperm function. *Biol Lett* 2006; 2: 246–9.
62 Anderson MJ, Nyholt J, Dixson AF. Sperm competition and the evolution of sperm midpiece volume in mammals. *J Zool* 2005; 267: 135.
63 Gomendio M, Roldan ER. Implications of diversity in sperm size and function for sperm competition and fertility. *Int J Dev Biol* 2008; 52: 439–47.
64 Mossman JA, Pearson JT, Moore HD, Pacey AA. Variation in mean human sperm length is linked with semen characteristics. *Hum Reprod* 2013; 28: 22–32.
65 Holt WV, Hernandez M, Warrell L, Satake N. The long and the short of sperm selection *in vitro* and *in vivo*: swim-up techniques select for the longer and faster swimming mammalian sperm. *J Evol Biol* 2010; 23: 598–608.
66 Yaniz JL, Palacin I, Vicente-Fiel S, Sanchez-Nadal JA, Santolaria P. Sperm population structure in high and low field fertility rams. *Anim Reprod Sci* 2015; 156: 128–34.
67 Smith TT, Yanagimachi R. The viability of hamster spermatozoa stored in the isthmus of the oviduct: the importance of sperm-epithelium contact for sperm survival. *Biol Reprod* 1990; 42: 450–7.
68 Kotarska K, Lenartowicz M. Sperm migration and selection in the reproductive tract of female mice is mostly affected by male genotype. *Folia Biol (Krakow)* 2011; 59: 71–5.
69 Savage A, Williams WL, Fowler NM. A statistical study of head length variability of bovine spermatozoa and its application to the determination of fertility. *Trans Roy Soc Can Sect V* 1927; 21: 425–50.
70 Pant KP, Mukherjee DP. The effect of seasons on the sperm dimensions of buffalo bulls. *J Reprod Fertil* 1972; 29: 425–9.
71 Waheed MM, Ghoneim IM, Abdou MS. Morphometric characteristics of spermatozoa in the Arabian horse with regard to season, age, sperm concentration, and fertility. *J Equine Vet Sci* 2015; 35: 244–9.
72 Beatty RA, Mukherjee DP. Spermatozoan characteristics in mice of different ages. *J Reprod Fertil* 1963; 6: 261–8.
73 Saoud NB, Thomas DL, Hohenboken WD. Breed and sire effects on crossbred lamb production from Panama ewes and on spermatozoan midpiece length. *J Anim Sci* 1984; 59: 29–38.
74 Sharlin JS, Shaffner CS, Gee GF. Sperm head length as a predictor of fecundity in the sandhill crane, *Grus canadensis*. *J Reprod Fertil* 1979; 55: 411–3.
75 Beletti ME, Costa Lda F, Viana MP. A comparison of morphometric characteristics of sperm from fertile *Bos taurus* and *Bos indicus* bulls in Brazil. *Anim Reprod Sci* 2005; 85: 105–16.
76 Saravia F, Nunez-Martinez I, Moran JM, Soler C, Muriel A, *et al*. Differences in boar sperm head shape and dimensions recorded by computer-assisted sperm morphometry are not related to chromatin integrity. *Theriogenology* 2007; 68: 196–203.
77 Marti JI, Aparicio IM, Garcia-Herreros M. Head morphometric changes in cryopreserved ram spermatozoa are related to sexual maturity. *Theriogenology* 2011; 75: 473–81.
78 Boersma AA, Braun J, Stolla R. Influence of random factors and two different staining procedures on computer-assisted sperm head morphometry in bulls. *Reprod Domest Anim* 1999; 34: 77–82.
79 de Paz P, Mata-Campuzano M, Tizado EJ, Alvarez M, Alvarez-Rodriguez M, *et al*. The relationship between ram sperm head morphometry and fertility depends on the procedures of acquisition and analysis used. *Theriogenology* 2011; 76: 1313–25.
80 Katz DF, Overstreet JW, Samuels SJ, Niswander PW, Bloom TD, *et al*. Morphometric analysis of spermatozoa in the assessment of human male fertility. *J Androl* 1986; 7: 203–10.
81 Yamamoto Y, Maenosono S, Okada H, Miyagawa I, Sofikitis N. Comparisons of sperm quality, morphometry and function among human sperm populations recovered via SpermPrep II filtration, swim-up and Percoll density gradient methods. *Andrologia* 1997; 29: 303–10.
82 Maroto-Morales A, Ramon M, Garcia-Alvarez O, Montoro V, Soler AJ, *et al*. Sperm head phenotype and male fertility in ram semen. *Theriogenology* 2015; 84: 1536-41.
83 Savage A, Williams WL, Fowler NM. A Study of the head length variability of equine spermatozoa. *Can J Res* 1930; 3: 327–35.
84 Ostermeier GC, Sargeant GA, Yandell TB, Parrish JJ. Measurement of bovine sperm nuclear shape using Fourier harmonic amplitudes. *J Androl* 2001; 22: 584–94.
85 Ostermeier GC, Sargeant GA, Yandell BS, Evenson DP, Parrish JJ. Relationship of bull fertility to sperm nuclear shape. *J Androl* 2001; 22: 595–603.
86 Sailer BL, Jost LK, Evenson DP. Bull sperm head morphometry related to abnormal chromatin structure and fertility. *Cytometry* 1996; 24: 167–73.
87 Gravance CG, Casey ME, Casey PJ. Pre-freeze bull sperm head morphometry related to post-thaw fertility. *Anim Reprod Sci* 2009; 114: 81–8.
88 Jeyendran RS, Schrader SM, van der Ven HH, Burg J, Perez-Pelaez M, *et al*. Association of the *in-vitro* fertilizing capacity of human spermatozoa with sperm morphology as assessed by three classification systems. *Hum Reprod* 1986; 1: 305–8.
89 DeStefano F, Annest JL, Kresnow MJ, Schrader SM, Katz DF. Semen characteristics of Vietnam veterans. *Reprod Toxicol* 1989; 3: 165–73.
90 Boyle CA, Khoury MJ, Katz DF, Annest JL, Kresnow MJ, *et al*. The relation of computer-based measures of sperm morphology and motility to male infertility. *Epidemiology* 1992; 3: 239–46.
91 Soler C, Gaßner P, Nieschlag E, de Montserrat JJ, Gutiérrez R, *et al*. Use of the integrated semen analysis system (ISAS)® for morphometric analysis and its role in assisted reproduction technologies. *Rev Int Androl* 2005; 3: 112–9.
92 Casey PJ, Gravance CG, Davis RO, Chabot DD, Liu IK. Morphometric differences in sperm head dimensions of fertile and subfertile stallions. *Theriogenology* 1997; 47: 575–82.
93 Gravance CG, Liu IK, Davis RO, Hughes JP, Casey PJ. Quantification of normal head morphometry of stallion spermatozoa. *J Reprod Fertil* 1996; 108: 41–6.
94 Kwon WS, Oh SA, Kim YJ, Rahman MS, Park YJ, *et al*. Proteomic approaches for profiling negative fertility markers in inferior boar spermatozoa. *Sci Rep* 2015; 5: 13821.
95 Hirai M, Boersma A, Hoeflich A, Wolf E, Foll J, *et al*. Objectively measured sperm motility and sperm head morphometry in boars (*Sus scrofa*): relation to fertility and seminal plasma growth factors. *J Androl* 2001; 22: 104–10.
96 Lavara R, Vicente JS, Baselga M. Genetic variation in head morphometry of rabbit sperm. *Theriogenology* 2013; 80: 313–8.
97 Marco M, Balasch S, Vicente JS, Lavara R, Viudes-de-Castro MP, *et al*. Detection of sub-fertile rabbits by means of sperm head morphometric analysis. *J Androl* 2005: 80.
98 Marco-Jimenez F, Vicente JS, Lavara R, Balasch S, Viudes-de-Castro MP. Poor prediction value of sperm head morphometry for fertility and litter size in rabbit. *Reprod Domest Anim* 2010; 45: e118–23.
99 Gage MJ. Mammalian sperm morphometry. *Proc Biol Sci* 1998; 265: 97–103.
100 Gil MC, Garcia-Herreros M, Baron FJ, Aparicio IM, Santos AJ, *et al*. Morphometry of porcine spermatozoa and its functional significance in relation with the motility parameters in fresh semen. *Theriogenology* 2009; 71: 254–63.
101 Vernocchi V, Morselli MG, Lange Consiglio A, Faustini M, Luvoni GC. DNA fragmentation and sperm head morphometry in cat epididymal spermatozoa. *Theriogenology* 2014; 82: 982–7.
102 Nunez-Martinez I, Moran JM, Pena FJ. Identification of sperm morphometric subpopulations in the canine ejaculate: do they reflect different subpopulations in sperm chromatin integrity? *Zygote* 2007; 15: 257–66.
103 Marco-Jimenez F, Viudes-de-Castro MP, Balasch S, Moce E, Silvestre MA, *et al*. Morphometric changes in goat sperm heads induced by cryopreservation. *Cryobiology* 2006; 52: 295–304.
104 Garcia-Herreros M, Leal CL. Sperm morphometry: a tool for detecting biophysical changes associated with viability in cryopreserved bovine spermatozoa. *Andrologia* 2014; 46: 820–2.
105 Nunez-Martinez I, Moran JM, Pena FJ. Sperm indexes obtained using computer-assisted morphometry provide a forecast of the freezability of canine sperm. *Int J Androl* 2007; 30: 182–9.
106 Hidalgo M, Rodriguez I, Dorado JM. The effect of cryopreservation on sperm head morphometry in Florida male goat related to sperm freezability. *Anim Reprod Sci* 2007; 100: 61–72.
107 Gravance CG, White C, Robertson KR, Champion ZJ, Casey PJ. The effects of cryopreservation on the morphometric dimensions of caprine sperm heads. *Anim Reprod Sci* 1997; 49: 37–43.
108 Rubio-Guillen J, Gonzalez D, Garde JJ, Esteso MC, Fernandez-Santos MR, *et al*. Effects of cryopreservation on bull spermatozoa distribution in morphometrically distinct subpopulations. *Reprod Domest Anim* 2007; 42: 354–7.

Supplementary Table 2: Domestic mammalian species included in Table 2

Species	*Family*	*Order*
Bison bison	Bovidae	Artiodactyla
Bos taurus	Bovidae	Artiodactyla
Bubalus bubalis	Bovidae	Artiodactyla
Budorcas taxicolor (sub. *tibetana*)	Bovidae	Artiodactyla
Capra hircus	Bovidae	Artiodactyla
Ovis aries	Bovidae	Artiodactyla
Camelus dromedarius	Camelidae	Artiodactyla
Lama glama	Camelidae	Artiodactyla
Cervus elaphus (sub. *bactrianus*)	Cervidae	Artiodactyla
Cervus elaphus (sub. *barbarus*)	Cervidae	Artiodactyla
Cervus elaphus (sub. *macneilli*)	Cervidae	Artiodactyla
Dama dama	Cervidae	Artiodactyla
Rangifer tarandus	Cervidae	Artiodactyla
Sus scrofa	Suidae	Artiodactyla
Canis familiaris	Canidae	Carnivora
Felis catus	Felidae	Carnivora
Mustela putorius (sub. *eversmanni*)	Mustelidae	Carnivora
Oryctolagus cuniculus	Leporidae	Lagomorpha
Equus asinus	Equidae	Perissodactyla
Equus caballus	Equidae	Perissodactyla
Homo sapiens	Hominidae	Primates
Cavia porcellus	Caviidae	Rodentia