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Morphometric differences in sperm subpopulations in frozen-thawed semen of two bovine subspecies

Diferencias morfométricas de las subpoblaciones espermáticas en semen descongelado de dos subespecies bovinas

Diferenças morfométricas de subpopulações de espermatozoides em sêmen descongelado de duas subespécies bovinas

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Abstract

[Objetive] The objective was to characterize different sperm subpopulations based on morphometric parameters of frozen-thawed semen in two bovine subspecies using a CASA system. **[Methodology]** The experiment was carried out at the Costa Rica Institute of Technology from May to December 2023. Spermatozoa from 10 bulls (five animals of each subspecies, *Bos taurus and Bos indicus*) were evaluated after thawing of the semen doses by an ISAS®v1, Computer-Assisted Sperm Analysis (CASA)-Morph system. Sub-populations of head morphometric spermatozoa were characterized using multivariate procedures such as principal components (PCs) analysis and clustering methods (k-means model) **[Results]** The ejaculate of male exhibits significant heterogeneity and comprises diverse sperm subpopulations with differing morphometric patterns. Three different sperm subpopulations were identified from three PCs: head size, head shape, and symmetry of the sperm head and the degree to which it was pyriform. The proportions of the different sperm subpopulations varied in the two-bovine subspecies; *Bos taurus and*

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Bos indicus. Results indicated that subpopulations SP1, SP2, and SP3 were different for PC criteria and these differences were relevant. The variability in sperm morphometry assessments underscores the need to standardize semen evaluation protocols **[Conclusions]** These findings highlight the importance of knowing the diversity in sperm morphometry between subspecies to both improve in vitro evaluation tests and to distinguish subspecies.

Keywords: Biology; cattle; spermatozoa; subspecies; morphometry analysis; subpopulation

Resumen

[Objetivo] El objetivo fue la caracterización de diferentes subpoblaciones basada en parámetros morfométricos de semen descongelado de dos subespecies de ganado, utilizando un sistema CASA. **[Metodología]** El experimento se realizó en el Instituto Tecnológico de Costa Rica (ITCR), entre los meses de mayo y diciembre de 2023. Se analizaron dosis seminales de 10 toros (5 animales por cada subespecie, *Bos taurus* y *Bos indicus* y fueron evaluados luego de la descongelación de las dosis seminales usando ISAS®v1, CASA-Sistema de morfometría. Se empleó análisis multivariado a partir de componentes principales (PC) y métodos de agrupación (k-means model) por medio de las evaluaciones de la morfometría de la cabeza. **[Resultados]** El eyaculado de los toros exhibe una heterogeneidad significativa y comprende diversas subpoblaciones de espermatozoides con diferentes patrones morfométricos. Se identificaron tres subpoblaciones diferentes de espermatozoides a partir de tres PC asociados a: tamaño, forma y simetría de la cabeza y el grado en que eran piriformes. Las proporciones de las subpoblaciones encontradas de espermatozoides variaron en las dos subespecies bovinas: *Bos taurus* y *Bos indicus*. SP1, SP2 y SP3 (subpoblaciones) fueron diferentes para los criterios de los PC y las variaciones fueron importantes. La variabilidad en las evaluaciones de la morfometría espermática ratifica la necesidad de estandarizar los protocolos de evaluación del semen. **[Conclusiones]** Estos hallazgos resaltan la importancia de conocer la diversidad en la morfometría espermática entre subespecies, tanto para mejorar las pruebas de evaluación in vitro, como para distinguir las subespecies.

Palabras claves: Biología; Ganado; espermatozoide; subespecie; análisis de morfometría; subpoblación.

Resumo

[Objetivo] O objetivo foi a caracterização de diferentes subpopulações com base em parâmetros morfométricos de sêmen descongelado de duas subespécies de bovinos utilizando um sistema CASA. **[Metodologia]** O experimento foi realizado no Instituto Tecnológico da Costa Rica (ITCR), entre os meses de maio e dezembro de 2023. Foram analisadas doses de sêmen de 10 touros (5 animais para cada subespécie, *Bos taurus* e *Bos indicus* e foram avaliados após o descongelamento das doses seminais usando ISAS®v1, CASA-Sistema de morfometria. Utilizou-se análise multivariada a partir de componentes principais (PC) e métodos de agrupamento (modelo k-means) por meio das avaliações da morfometria da cabeça. **[Resultados]** A ejaculação dos touros exibe heterogeneidade significativa e compreende várias subpopulações de espermatozoides com diferentes padrões morfométricos. Três subpopulações diferentes de espermatozoides foram identificadas a partir de três PCs associados a: tamanho, forma e simetria da cabeça e o grau em que eram piriformes. As proporções das subpopulações de espermatozoides encontradas variaram nas duas subespécies bovinas; *Bos taurus* e *Bos indicus*. SP1, SP2 e SP3 (subpopulações) foram diferentes para os critérios de PC e as variações foram significativas. A variabilidade nas avaliações da

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morfometria espermática ratifica a necessidade de padronizar os protocolos de avaliação do sêmen. **[Conclusões]** Essas descobertas destacam a importância de conhecer a diversidade na morfometria espermática entre as subespécies, tanto para melhorar os testes de avaliação in vitro quanto para distinguir as subespécies.

Palavras-chave: biologia; gado; espermatozoide; subespécie; análise morfométrica; subpopulação.

Introduction

The reproductive study of animals used in the livestock industry is part of the success of these systems (Madhusoodan *et al*., 2019). Bull fertility is important in the development of the productive systems (Waberski *et al*. 2021, Barth, 2018), and their evaluation allows one to select those animals with better reproductive conditions (Petrunkina *et al*., 2007) or discard animals or ejaculates with poor quality parameters that could be infertile or subfertile. A bull's seminal quality could be influenced by multiple factors, such as season (Barth, 2018), health (Taylor *et al*., 2018), nutrition (Cheah & Yang, 2011), animal species (Castellini *et al*., 2011), and semen conservation (Awad, 2011).

Bull semen preservation is usually associated with assisted reproduction techniques in which the spermatozoon is generally frozen. However, subjecting the cells to this process can affect their characteristics, this limiting their functionality when they are subsequently thawed (Awad, 2011). Sperm morphometry is a cell characteristic that can be used as an indicator for potential freezability or male fertility (Maroto-Morales *et al*., 2016; Villaverde-Morcillo *et al*., 2017).

Sperm morphometry is usually determined by performing the CASA-Morph module (Gallagher *et al*., 2018; Mortimer & De Jonge, 2018; Valverde *et al*., 2019a; 2019b), which allows one to analyze characteristics related to the size and shape of the head, acrosome, and intermediate piece of the sperm (Vicente-Fiel *et al*., 2013; Yániz *et al*., 2015). These characteristics may differ according to the method of cell fixation (Soler *et al*., 2015), staining (Banaszewska *et al*., 2015; Boersma *et al*., 1999), and evaluation systems (Vicente-Fiel *et al*., 2013). Morphometry is relevant because it could be used to predict the fertility potential of males (Franken, 2015; Toner *et al*., 1995) and to explain alterations of cells caused by the spermiogenesis process (Valverde *et al*., 2016), as well as explain some relationships with other sperm variables (Giaretta *et al*., 2017; Hook & Fisher, 2020). Despite the importance of this analysis, its use has been characterized by variability in evaluation and methodologies (Garcia-Herreros *et al*., 2006).

For this reason, these variations in the morphometry of sperm cells have been analyzed and distinct characteristics have been determined in the ejaculate of the same animal (Buchelly Imbachí *et al*., 2022; Martínez-Pastor, 2022). Therefore, using cluster analysis allowed us to identify different subpopulations with an ejaculate based on the data from the CASA systems (Barquero *et al*., 2021; Martínez-Pastor, 2022; Valverde *et al*., 2016; Yániz *et al*., 2018). These new analyses have favored the explanation of the conformation of the ejaculates and their relationship with the

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potential fertility of the animals analyzed (Santolaria *et al*., 2015).

On the other hand, sperm morphometric characterization could be used to distinguish animal species (Sampaio *et al*., 2017). Sperm morphometry differences have been observed when comparing different species of foxes (Andraszek *et al*., 2020), ovine (Martínez-Fresneda *et al*., 2019), neotropical primate (Sampaio *et al*., 2017), and even dog breeds (Soler *et al*., 2017a). In the literature, there are separate studies of sperm morphometry for both *B. taurus* and *B. indicus*, but there are only a few that compare them within the same study (Beletti *et al*., 2005) .To our knowledge, no study has compared sperm morphometry between *B. taurus taurus* and *B. taurus indicus* using a CASA-morph system and subpopulation analyses.

Therefore, the aim of this study was to determine different sperm subpopulations based on morphometry parameters of frozen-thawed semen in two bovine subspecies using a CASA-morph system.

Methodology

The study was conducted using cryopreserved straws and doses were supplied by Avance Genético S.A. (Alajuela, Costa Rica). The study was conducted from May to December 2023, at the Animal Reproduction Laboratory (AndroTEC), located at the Campus Tecnológico Local San Carlos (CTLSC), in Santa Clara, Florencia, Alajuela, Costa Rica (CRTM05; X: 444296 Y: 1146016). Ethical approval was granted by the Committee of Centro de Investigación y Desarrollo de la Agricultura Sostenible para el Trópico Húmedo at the Costa Rica Institute of Technology (CIDASTH-ITCR) under Section 20/2023, Article 1.0, reference DAGSC-188-2023 and CIE-206-2023 Section 20-2023, Article 3.19.

Animals, semen collection and processing: Semen was collected from 5 Simmental (*Bos taurus*) bulls and 5 Brahman (*Bos indicus*) bulls with an average age of 5.7±2.8 and 3.6±1.6 years respectively, by artificial vagina under a collection program of two times per week. The semen samples were assessed for volume by conical tube graduated at 0.1 mL and concentration using a bovine photometer Accucell (*IMV, L'Aigle, France*) at 530 nm wavelength. Semen was diluted using a commercial extender OptiXcell® (*IMV, L'Aigle, France),* with a ratio depending on ejaculate volume. In any case, the final concentration given with the diluent was 25×10^6 cells/straw. The equilibrate procedure consist in a decrease slowly at a rate of $-0.3°min^{-1}$ until 4 °C of the diluted semen, after these were maintained at the same temperature for 4–5 hours. The semen was packed in 0.25 mL straws with an automatic filling and sealing machine (MRS1, IMV Technologies, L'Aigle, France). Then, the straws were frozen in a programmable freezer, (*Digitcool 5300, IMV, L'Aigle, France*) at the following rates: 4 to −10°C at 5°C min^{-1}, −10 to −100°C at 40°C min^{-1}, and −110 to −140°C at 20°C min^{-1}. Finally, the straws were stored by plunging into liquid nitrogen.

Sperm sample preparation for morphometric analysis: Sperm morphometric analysis was performed slides from straws for each bull. In a slide a previously mixed subsample of 10 µL extended were placed, extended and identified. The samples were stained with the Diff-Quik® kit (*Medion Diagnostics, Düdingen, Switzerland*) following all the manufacturer's instructions step by step. The slides were then kept for the subsequent double-blind scheme analysis.

Morphometric assessment systems: Morphometric evaluations of semen were performed using the ISAS®v1

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(*Integrated Semen Analysis System*, Proiser R+D, Valencia, Spain) using the parameters that the software brings by default. The images capture was made using a digital video camera (Proiser 782 m, Proiser R + D) connected to a UB203 microscope (UOP/Proiser R + D) with a 100× bright-field objective and an attached 3.3× photo-ocular. The camera video frame grabber array size was 746 × 578 × 8 bit, using 256 gray levels with 0.084 µm/pixel resolution available in both axes of the analysis images. A minimum of 200 images of different spermatozoa were captured and analyzed. The spermatozoon was capture randomly, discriminated these if there were particles that potentially interfered with the sperm head boundary. In case of an erroneous definition of the sperm head boundary, the system allowed us to vary the analysis factor and if not was possible to analyze a correct boundary, the cell was deleted from the analysis. A graphical schematic of the experimental design is shown in Figure 1.

Morphometric variables: Eight sperm head morphometric variables were analyzed, divided into four parameters of head size (length [L, µm], width [W, µm], area [A, µm^2], and perimeter [P, µm]) and four head shape parameters derived from the head size (ellipticity [L/W], rugosity [$4\pi A/P^2$], elongation [(L−W)/(L+W)], and regularity [$\pi LW/4A$]) were also obtained. All the data obtained from each sperm were saved in an Excel® (Microsoft Corporation, Redmond, Washington, USA) for further analysis.

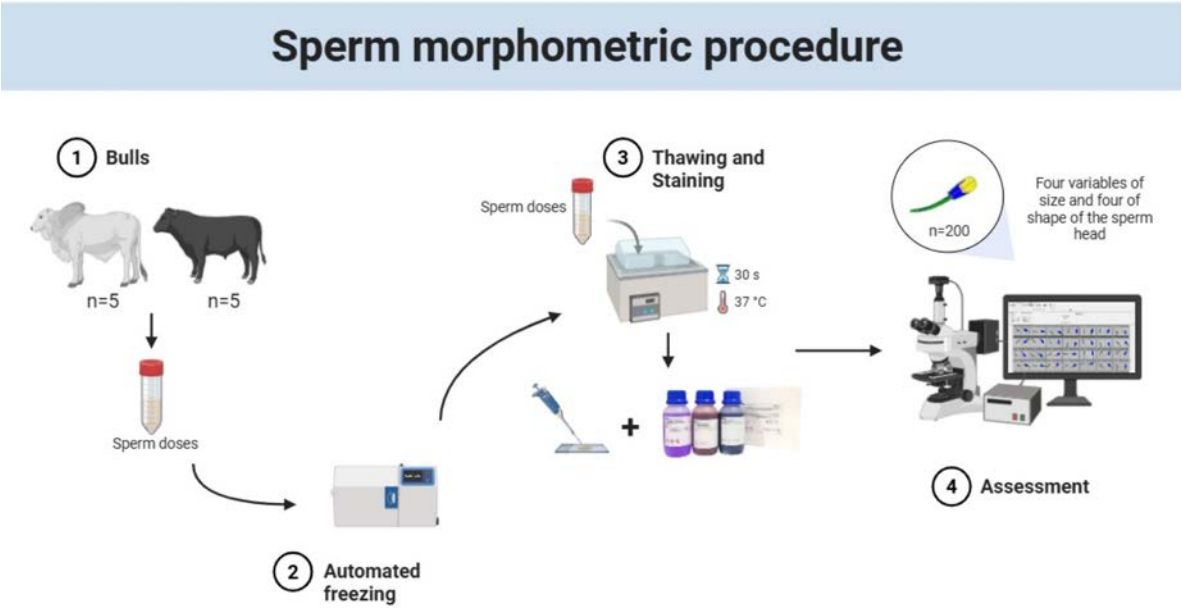

Figure 1. Bovine sperm morphometric assessment procedure. (1) Ejaculates were obtained from two bull groups (n = 5 per group) and packaged as sperm doses. (2) Doses were cryopreserved using an automated freezing protocol. (3) For analysis, frozen doses were thawed (30 s at 37 °C), smeared on slides, and stained with the morphometric stain described in the Methods. (4) At least 200 sperm heads per sample were digitally imaged and analyzed using a computer-assisted morphometry system to generate four size and four shape descriptors of the sperm head (see Materials and Methods for parameter definitions).

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Statistical analysis: Data for all sperm variables were assessed for homoscedasticity using Levene tests and for normality using a normal probability plot. An identity link function with a normal distribution was assumed for all sperm morphometric response variables. ANOVA was applied to evaluate statistical differences between treatments for these variables. Post-hoc pairwise comparisons between species means were conducted using the Tukey–Kramer test.

Multivariate procedures: Multivariate procedures were employed to identify sperm clusters from the subset of sperm morphometric data. To mitigate scaling effects, all morphometric variables were standardized. Principal factor analysis (PFA) was conducted to derive a few linear combinations that retained most variance of the original variables' information. Prior communalities were estimated using the maximum absolute correlation coefficient between each variable and others. The number of principal factors (PFs) extracted was determined using the Kaiser criterion, selecting those with eigenvalues >1. The dataset's adequacy for factor extraction was assessed using the KMO (Kaiser–Meyer–Olkin) statistic (Spencer, 2013). Varimax rotation with Kaiser normalization (Kaiser, 1958) was applied.

A two-step cluster procedure was performed using the sperm-derived indices obtained from the principal component analysis (PCA). Sperm morphometric measurements within each ejaculate were clustered based on shape and size parameters using a non-hierarchical K-means clustering procedure with the Euclidean distance metric (Kaufman & Rousseeuw, 1991). The analysis identified sperm subpopulations and detected outliers. ANOVA was applied to evaluate statistical differences between clusters for all morphometric variables, with significance defined at $P < 0.05$. Pairwise comparisons between cluster means were conducted using the Tukey–Kramer test. Results are presented as mean ± standard error of the mean. All data were analyzed using IBM SPSS, version 23.0, for Windows (SPSS Inc., Chicago, IL, USA).

Analysis and results

Some previous research has demonstrated the adverse effects of cryopreservation on semen quality (Maroto-Morales *et al*., 2016). The cryopreservation process has an important impact on sperm morphology (Awad, 2011) and potential fertilization capacity (Franken, 2015), so it has a very specific effect on the proportion of spermatozoa that make up the different subpopulations (Rubio-Guillén *et al*., 2007). The decrease in the percentage of sperm with long and large sperm heads, and the increase in the proportion of cells in the smaller sperm subpopulation is a known consequence of cryopreservation (Gravance *et al*., 2009). This effect may directly condition the results observed in this work, based on the evidence that European breeds may have greater tolerance to cryopreservation processes (Saranholi *et al*., 2021). However, this factor would also imply that the decrease in the morphometric parameters of one subspecies compared to another causes an effect on the distribution of subpopulations, since, when evaluating other variables such as kinetics, some changes are observed as reported in previous studies (Víquez *et al*., 2020).

It has been reported that sperm morphometry can be affected by different factors, such as osmotic pressure, staining techniques, freezing and thawing (Caldeira

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et al., 2022; García-Molina *et al*., 2023; Soler *et al*., 2017a; Soler *et al*., 2017b; Soler *et al*., 2005, 2016; Soler *et al*., 2017c; Valverde et al., 2019; Yániz *et al*., 2016). These variables can have a significant impact not only on sperm dimensions (Awad, 2011), which could lead to inaccurate assessment, but also on chromatic structure, which can have direct negative implications on the individual's fertility potential (Banaszewska *et al*., 2015). Results of this work allow us to understand more about the variations that could occur in the morphometric characteristics of spermatozoa between the different bovine subspecies.

Differences between species indicated that genotype composition influenced the value of each sperm head size and shape variable. When the sperm head length was studied, samples from *B. taurus* (9.007 ± 0.037 µm) were the longest ($p < 0.05$) compared to those obtained from *B. indicus* (8.969 ± 0.026 µm). Width values differed ($p < 0.05$) according to species, in decreasing order, of *B. taurus* (4.886 ± 0.016 µm), and *B. indicus* (4.756 ± 0.011 µm). The head area of spermatozoa in both *B. taurus* or *B. indicus* was not different ($p > 0.05$). There were differences in head sperm perimeters ($p < 0.05$) when comparing the use of *B. taurus* and *B. indicus* species. In relation to head shape variables, the values oscillated differently in both species (Table 1).

Heterogeneity in mammalian ejaculates could explain the behavior of species and their evolution according to the conditions in which they have developed (Holt & Van Look, 2004; Ramón *et al*., 2014) as this has allowed us to understand how different types of spermatozoa different functions within the ejaculate could have to achieve successful reproduction of the species (Ramón *et al*., 2014). Morphometric evaluations between the subspecies *Bos taurus* and *Bos indicus* showed differences in sperm size and shape characteristics. This coincides with previous research that has determined that sperm from zebu bulls tend to be smaller in size compared to other breeds, including European breeds (Beletti *et al*., 2005).

Three PC factors were identified. In *B. indicus*, PC1 was found to be positively associated with ellipticity and elongation, and negatively related with the rugosity parameter. PC2 was related to perimeter and area. PC3 was related to shape variable regularity. In the B. taurus, PC1 was found to

Table 1. *Morphometric variables (mean ± SEM) of bull sperm size and head shape of in two different bovine subspecies.*

Variables	*Bos indicus*	*Bos taurus*
Length	8.969 ± 0.026^{b}	9.007 ± 0.037^{a}
Width	4.756 ± 0.011^{b}	4.886 ± 0.016^{a}
Area	37.120 ± 0.124	37.792 ± 0.172
Perimeter	24.767 ± 0.048^{b}	24.791 ± 0.068^{a}
Ellipticity	1.896 ± 0.006^{a}	1.846 ± 0.009^{b}
Rugosity	0.761 ± 0.001^{b}	0.772 ± 0.002^{a}
Elongation	0.307 ± 0.002^{a}	0.296 ± 0.002^{b}
Regularity	0.901 ± 0.002^{b}	0.915 ± 0.003^{a}

Note: SEM: standard error of the mean. Length (L, µm), width (W, µm), area (A, μm^2), perimeter (P, µm), ellipticity (L/W), rugosity ($4\pi A/P^2$), elongation ((L − W)/(L + W)), regularity ($\pi LW/4A$). $^{a–b}$ Different letters indicate differences between ejaculate sub-populations for morphometric variables. $p < 0.05$. Source: own study.

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be related to head size (area, width, and perimeter, in decreasing order) and PC2 was associated with shape head (ellipticity, elongation and regularity and, to a lesser degree, length). PC3 was negatively related with regularity. The total variance explained was 95.39 %, and 90.798 % for *B. indicus* and *B. taurus*, respectively (Table 2).

This discovery underscores the importance of further investigating the genetic mechanisms responsible for these differences for a thorough understanding of how breed genetics can shape sperm characteristics as a measure of adaptation. These results suggest that the cryopreserved materials of *Bos indicus* and *Bos taurus* could have three sperm subpopulations according to their shape and size. Other researchers have studied the relationship that the shape and size of sperm could have with evolutionary mechanisms of different species (Kramer *et al*., 2024). In addition, other studies found in the literature comparing the morphometric characteristics of *Bos taurus* and *Bos indicus* and suggest that the differences could be associated with individual characteristics of the animals and their fertility (Beletti *et al*., 2005) and that they could be related as a function of membrane and acrosome quality (Palacin *et al*., 2020).

Principal components identify three subpopulations in *B. indicus*: SP1 with 32.9 % of the total spermatozoa, SP2 with 43.0 % of the cell analyzed in this species, and SP3 with 24.1 % of the total spermatozoa. For *B. taurus*, three subpopulations were found: SP1 contained 46.0 % of the sperm cells. SP2 had a total of 32.0 % of the male gamete. The last subpopulation (SP3) was comprised of 22.0 % cells (Figure 2).

The distribution was not the same for each species but there were differences in subpopulations within each species ($p < 0.05$), and the distribution was not the same for each species. Results from *B. indicus* indicated differences between subpopulations upon comparison. SP1 was characterized by the medium size (length and area), while SP2 was characterized by the smallest cells and SP3 showed the highest spermatozoa size. Similarly, results described in *B. taurus* indicate that sperm subpopulations were

Table 2. *Eigenvectors of principal components (PCs)* for the morphometric variables of bull sperm size and head shape in bovine subspecies.*

Variable	*Bos indicus*			*Bos taurus*		
	PC1	**PC2**	**PC3**	**PC1**	**PC2**	**PC3**
Length	0.703				0.765	
Width				0.898		
Area		0.929		0.949		
Perimeter		0.915		0.892		
Ellipticity	0.983				0.810	
Elongation	0.984				0.808	
Rugosity	-0.886					
Regularity			0.995		0.804	-0.828
Var Exp	52.772	27.114	15.503	36.317	32.628	21.853

Note: Var Exp: variance explained in each PC. Total variance explained: *Bos indicus* = 95.389 %; *Bos taurus* = 90.798 %. *Indicates the more important variables in each PC. Only eigenvectors > 0.6 are presented. Source: own study.

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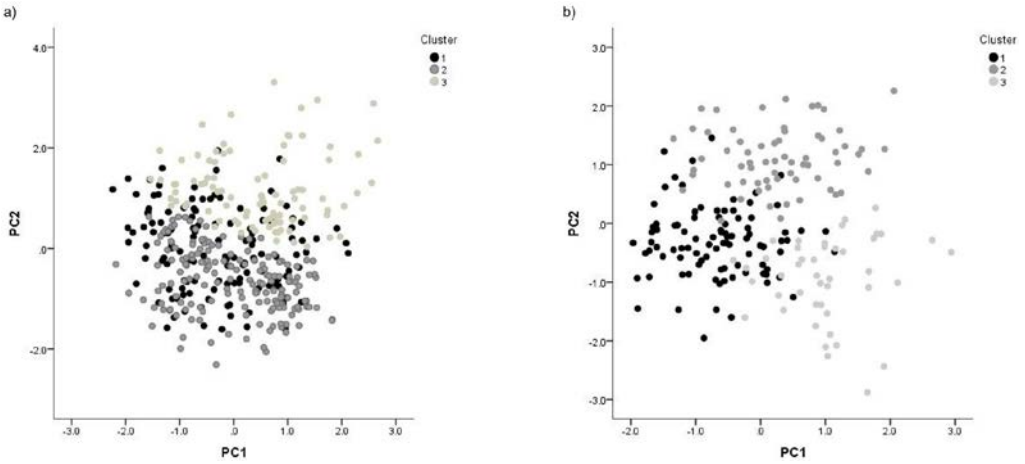

Figure 2. *Interactions between principal components (PCs) for the morphometric variables of bull sperm size and head shape by bovine subspecies. a) Bos indicus, b) Bos taurus.*
Note. Source: own study.

different ($p < 0.05$). SP1 was characterized by the smallest cells, and the head shape was the intermediate for all variables, except for the rugosity value, which was the highest. SP2 and SP3 were high, with the highest values of each variable oscillating from one to the other. In both species, a different composition was observed, with SP3 having the highest head size values, except for the length. The shape variables in *B. taurus* indicate that SP1 and SP2 values were different to SP2 and SP3 for *B. indicus* (Table 3).

Cluster analysis has shown that bull ejaculates are not homogeneous in terms of sperm population (Martínez-Pastor, 2022), and the distribution of spermatozoa also varied between subspecies. Results showed the presence of subpopulations with wide variability in both *B. taurus* and *B. indicus* bulls. These findings suggest that the

Table 3. *Morphometry of sperm heads and head shape variables (mean ± SEM) of bull semen sub-populations (SPs) in different bovine subspecies.*

Variables	*Bos indicus*			*Bos taurus*		
	SP1	**SP2**	**SP3**	**SP1**	**SP2**	**SP3**
Length	9.110 ± 0.031^{ar}	8.634 ± 0.027^{bt}	9.273 ± 0.036^{cx}	8.581 ± 0.040^{as}	9.768 ± 0.048^{bu}	9.009 ± 0.057^{cy}
Width	4.888 ± 0.016^{ar}	4.678 ± 0.013^{ct}	4.821 ± 0.018^{bx}	4.726 ± 0.023^{as}	4.829 ± 0.028^{bu}	5.068 ± 0.034^{cy}
Area	37.272 ± 0.126^{ar}	35.941 ± 0.109^{bt}	39.381 ± 0.147^{bx}	35.232 ± 0.240^{as}	39.068 ± 0.288^{bu}	40.485 ± 0.347^{cy}
Perimeter	24.733 ± 0.050^{ar}	24.246 ± 0.043^{bt}	25.678 ± 0.058^{cx}	23.851 ± 0.082^{as}	25.532 ± 0.098^{bu}	25.815 ± 0.118^{bx}
Ellipticity	1.875 ± 0.010^{br}	1.856 ± 0.009^{at}	1.934 ± 0.012^{cx}	1.818 ± 0.009^{as}	2.025 ± 0.011^{bu}	1.780 ± 0.013^{cy}
Rugosity	0.766 ± 0.002^{br}	0.768 ± 0.002^{ct}	0.752 ± 0.002^{ax}	0.778 ± 0.003^{as}	0.753 ± 0.003^{bu}	0.764 ± 0.004^{cy}
Elongation	0.301 ± 0.002^{br}	0.297 ± 0.002^{at}	0.315 ± 0.003^{cx}	0.290 ± 0.002^{as}	0.338 ± 0.003^{bu}	0.280 ± 0.003^{cy}
Regularity	0.937 ± 0.002^{ar}	0.881 ± 0.002^{bt}	0.889 ± 0.002^{cx}	0.904 ± 0.003^{as}	0.949 ± 0.004^{bu}	0.886 ± 0.005^{cx}

Note: SEM.: standard error of the mean. Length (L, µm), width (W, µm), area (A, µm^2), perimeter (P, µm), ellipticity (L/W), rugosity ($4\pi A/P^2$), elongation ((L − W)/(L + W)), regularity ($\pi LW/4A$). $^{a–c}$ Different letters within species indicate differences between ejaculate sub-populations for morphometric variables. $^{r-s}$ Different letters within SP1; $^{t-u}$ within SP2; and $^{x-y}$ within SP3 indicate differences between bull species for morphometric variables. $p < 0.05$. Source: own study.

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differences could be related to genetics between these two subspecies, evidence that points towards the possibility that race-associated genetics could exert a significant influence on the observed variations in sperm morphometry (Terán *et al*., 2021; Thurston *et al*., 2002). Despite this, it is necessary to further investigate the possible implications of these genetic differences and their relevance on other sperm variables as an adaptation mechanism of each subspecies to conditions that affect the reproductive success of livestock.

Conclusions

The variability in semen morphometry analysis underscores the need to standardize semen evaluation protocols. This approach has revealed differences in sperm characteristics between subspecies, such as *B. taurus* and *B. indicus*, indicating potential genetic influences. Furthermore, the cryopreservation process itself introduces changes in sperm morphology, which begs for a comprehensive understanding of its effects on different sperm subpopulations.

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Conflict of Interest

The authors declare no conflict of interest.

Author contribution statement

Conceptualization, A.V., M.A.S; methodology, F.S., L.V., A.V.; software, F.S., L.V., J.S., I.A.Z.; validation, F.S, A.V.; formal analysis, A.V.; investigation, F.S., L.V., I.A.Z.; resources, A.V.; data curation, F.S., L.V., , I.A.Z; writing—original draft preparation, F.S., A.V., I.A.Z.; writing—review and editing, F.S., A.V., J.S., M.B., M.A.S., C.D.C.; visualization, A.V.; supervision, A.V.; project administration, A.V.; funding acquisition, A.V., M.A.S.. All authors have read and agreed to the published version of the manuscript.

Data availability statement

The data supporting the results of this study will be made available by the corresponding author, **[A.V.]**, upon reasonable request.

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References

Andraszek, K., Banaszewska, D., Szeleszczuk, O., Kuchta-gładysz, M., & Grzesiakowska, A. (2020). Morphometric Characteristics of the Spermatozoa of Blue Fox (*Alopex lagopus*) and Silver Fox (*Vulpes vulpes*). *Animals* , *10*(10), 1927. https://doi.org/10.3390/ANI10101927

Awad, M. M. (2011). Effect of some permeating cryoprotectants on CASA motility results in cryopreserved bull spermatozoa. *Animal Reproduction Science*, *123*(3-4), 157-162. https://doi.org/10.1016/j.anireprosci.2011.01.003

Banaszewska, D., Andraszek, K., Czubaszek, M., & Biesiada–Drzazga, B. (2015). The effect of selected staining techniques on bull sperm morphometry. *Animal Reproduction Science*, *159*, 17-24. https://doi.org/10.1016/j.anireprosci.2015.06.019

Barquero, V., Roldan, E. R. S., Soler, C., Yániz, J. L., Camacho, M., & Valverde, A. (2021). Predictive Capacity of Boar Sperm Morphometry and Morphometric Sub-Populations on Reproductive Success after Artificial Insemination. *Animals*, *11*(4), 920. https://doi.org/10.3390/ANI11040920

Barth, A. D. (2018). Review: The use of bull breeding soundness evaluation to identify subfertile and infertile bulls. *Animal*, *12*, s158-s164. https://doi.org/10.1017/S1751731118000538

Beletti, M. E., Costa, L. da F., & Viana, M. P. (2005). A comparison of morphometric characteristics of sperm from fertile *Bos taurus* and *Bos indicus* bulls in Brazil. *Animal Reproduction Science*, *85*(1-2), 105-116. https://doi.org/10.1016/j.anireprosci.2004.04.019

Boersma, A. A., Braun, J., & Stolla, R. (1999). Influence of Random Factors and Two Different Staining Procedures on Computer-assisted Sperm Head Morphometry in Bulls. *Reproduction in Domestic Animals*, *34*(2), 77-82. https://doi.org/10.1111/j.1439-0531.1999.tb01387.x

Buchelly Imbachí, F., Zalazar, L., Pastore, J. I., Nicolli, A., Ledesma, A., Hozbor, F. A., Cesari, A., & Ballarin, V. (2022). Clustering and classification software for sperm subpopulation analysis. *Computer Methods in Biomechanics and Biomedical Engineering: Imaging & Visualization*, *10*(6), 585-598. https://doi.org/10.1080/21681163.2021.2012831

Caldeira, C., Hernández-Ibánez, S., Vendrell, A., Valverde, A., García-Molina, A., Gallego, V., Asturiano, J. F., & Soler, C. (2022). Characterisation of European eel (*Anguilla anguilla*) spermatozoa morphometry using Trumorph tool in fixed and non-fixed samples. *Aquaculture*, *553*, 738047. https://doi.org/10.1016/J.AQUACULTURE.2022.738047

Castellini, C., Dal Bosco, A., Ruggeri, S., Collodel, G., Bosco, A. D., Ruggeri, S., & Collodel, G. (2011). What is the best frame rate for evaluation of sperm motility in different species by computer-assisted sperm analysis? *Fertility and Sterility, 96*(1), 24-27. https://doi.org/10.1016/j.fertnstert.2011.04.096

Cheah, Y., & Yang, W. (2011). Functions of essential nutrition for high quality spermatogenesis. *Advances in Bioscience and Biotechnology*, *2*(4), 182-197. https://doi.org/10.4236/abb.2011.24029

Franken, D. R. (2015). How accurate is sperm morphology as an indicator of sperm function? *Andrologia*, *47*(6), 720-723. https://doi.org/10.1111/AND.12324

Gallagher, M. T., Smith, D. J., & Kirkman-Brown, J. C. (2018). CASA: Tracking the past and plotting the future. *Reproduction, Fertility and Development, 30*(6), 867-874. https://doi.org/10.1071/RD17420

Garcia-Herreros, M., Aparicio, I. M., Baron, F. J., Garcia-Marin, L. J., & Gil, M. C. (2006). Standardization of sample preparation, staining and sampling methods for automated sperm head morphometry analysis of boar spermatozoa. *International Journal of Andrology*, *29*(5), 553-563. https://doi.org/10.1111/j.1365-2605.2006.00696.x

García-Molina, A., Navarro, N., Cerveró, C., Sadeghi, S., Valverde, A., Roldan, E. R. S., Bompart, D., Garrido, N., & Soler, C. (2023). Effect of incubation and analysis temperatures on sperm kinematics and morphometrics during human semen analysis. *Revista Internacional de Andrología*, *21*(2), 100350. https://doi.org/10.1016/J.ANDROL.2023.100350

DOI: https://dx.doi.org/10.15359/ru.39-1.10
E-ISSN: 2215-3470
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Giaretta, E., Munerato, M., Yeste, M., Galeati, G., Spinaci, M., Tamanini, C., Mari, G., & Bucci, D. (2017). Implementing an open-access CASA software for the assessment of stallion sperm motility: Relationship with other sperm quality parameters. *Animal Reproduction Science*, *176*, 11-19. https://doi.org/10.1016/j.anireprosci.2016.11.003

Gravance, C. G., Casey, M. E., & Casey, P. J. (2009). Pre-freeze bull sperm head morphometry related to post-thaw fertility. *Animal Reproduction Science*, *114*(1-3), 81-88. https://doi.org/10.1016/j.anireprosci.2008.09.014

Holt, W., & Van Look, K. (2004). Concepts in sperm heterogeneity, sperm selection and sperm competition as biological foundations for laboratory tests of semen quality. *Reproduction*, *127* (5), 527-535. https://doi.org/10.1530/rep.1.00134

Hook, K. A., & Fisher, H. S. (2020). Methodological considerations for examining the relationship between sperm morphology and motility. *Molecular Reproduction and Development*, *87*(6), 633-649. https://doi.org/10.1002/mrd.23346

Kaiser, H. F. (1958). The varimax criterion for analytic rotation in factor analysis. *Psychometrika*, *23*, 187-200. https://doi.org/10.1007/BF02289233

Kaufman, Leonard., & Rousseuw, P. J. (1991). Finding Groups in Data: An Introduction to Cluster Analysis. *Biometrics*, *47*(2), 788. https://doi.org/10.2307/2532178

Kramer, E. M., Enelamah, J., Fang, H., & Tayjasanant, P. A. (2024). Karyotype depends on sperm head morphology in some amniote groups. *Frontiers in Genetics*, *15,* 1396530. https://doi.org/10.3389/fgene.2024.1396530

Madhusoodan, A. P., Sejian, V., Rashamol, V. P., Savitha, S. T., Bagath, M., Krishnan, G., & Bhatta, R. (2019). Resilient capacity of cattle to environmental challenges – An updated review. *Journal of Animal Behaviour and Biometeorology*, *7*(3), 104-118. https://doi.org/10.31893/2318-1265jabb.v7n3p104-118

Maroto-Morales, A., García-Álvarez, O., Ramón, M., Martínez-Pastor, F., Fernández-Santos, Mr., Soler, Aj., & Garde, J. (2016). Current status and potential of morphometric sperm analysis. *Asian Journal of Andrology*, *18*(6), 863. https://doi.org/10.4103/1008-682X.187581

Martínez-Fresneda, L., O'Brien, E., Velázquez, R., Toledano-Díaz, A., Martínez-Cáceres, C. M., Tesfaye, D., Schellander, K., García-Vázquez, F. A., & Santiago-Moreno, J. (2019). Seasonal variation in sperm freezability associated with changes in testicular germinal epithelium in domestic (*Ovis aries)* and wild (*Ovis musimon*) sheep. *Reproduction, Fertility, and Development*, *31*(10), 1545-1557. https://doi.org/10.1071/RD18511

Martínez-Pastor, F. (2022). What is the importance of sperm subpopulations? *Animal Reproduction Science*, *246*, 106844. https://doi.org/10.1016/j.anireprosci.2021.106844

Mortimer, S. T., & De Jonge, C. J. (2018). CASA—Computer-Aided Sperm Analysis. *Encyclopedia of Reproduction*, *5,* 59-63. https://doi.org/10.1016/B978-0-12-801238-3.64935-8

Palacin, I., Santolaria, P., Alquezar-Baeta, C., Soler, C., Silvestre, M., & Yániz, J. (2020). Relationship of sperm plasma membrane and acrosomal integrities with sperm morphometry in *Bos taurus*. *Asian Journal of Andrology*, *22*(6), 578. https://doi.org/10.4103/aja.aja_2_20

Petrunkina, A. M., Waberski, D., Günzel-Apel, A. R., & Töpfer-Petersen, E. (2007). Determinants of sperm quality and fertility in domestic species. *Reproduction*, *134*, 3-17. https://doi.org/10.1530/REP-07-0046

Ramón, M., Jiménez-Rabadán, P., García-Álvarez, O., Maroto-Morales, A., Soler, A., Fernández-Santos, M., Pérez-Guzmán, M., & Garde, J. (2014). Understanding Sperm Heterogeneity: Biological and Practical Implications. *Reproduction in Domestic Animals*, *49*, 30-36. https://doi.org/10.1111/rda.12404

Rubio-Guillén, J., González, D., Garde, J., Esteso, M., Fernández-Santos, M., Rodríguez-Gíl, J., Madrid-Bury, N., & Quintero-Moreno, A. (2007). Effects of Cryopreservation on Bull Spermatozoa Distribution in Morphometrically Distinct Subpopulations. *Reproduction in Domestic Animals*, *42*(4), 354-357. https://doi.org/10.1111/j.1439-0531.2006.00788.x

Sampaio, W. V., Oliveira, K. G., Leão, D. L., Caldas-Bussiere, M. C., Queiroz, H. L., Paim, F. P., Santos, R. R., & Domingues, S. F. S. (2017). Morphologic analysis of sperm from two neotropical primate species: comparisons between the squirrel monkeys *Saimiri collinsi* and *Saimiri vanzolinii*. *Zygote*, *25*(2), 141-148. https://doi.org/10.1017/S0967199416000411

DOI: https://dx.doi.org/10.15359/ru.39-1.10
E-ISSN: 2215-3470
CC: BY-NC-ND

Santolaria, P., Vicente-Fiel, S., Palacín, I., Fantova, E., Blasco, M. E., Silvestre, M. A., & Yániz, J. L. (2015). Predictive capacity of sperm quality parameters and sperm subpopulations on field fertility after artificial insemination in sheep. *Animal Reproduction Science*, *163*, 82-88. https://doi.org/10.1016/j.anireprosci.2015.10.001

Saranholi, D. A. C., Paula, R. R. de, Pytilak, E., Afonso, F., Canela, L. F., Almeida, A. B. M. de, Hidalgo, M. M. T., Martins, M. I. M., Blaschi, W., & Barreiros, T. R. R. (2021). Comparison of seminal characteristics of Aberdeen Angus, Holstein and Nelore bulls before and after cryopreservation. *Research, Society and Development*, *10*(16), e408101623382. https://doi.org/10.33448/rsd-v10i16.23382

Soler, C., Alambiaga, A., Martí, M. A., García-Molina, A., Valverde, A., Contell, J., & Campos, M. (2017a). Dog sperm head morphometry: its diversity and evolution. *Asian Journal of Andrology*, *19*(2), 149-153. https://doi.org/10.4103/1008-682X.189207

Soler, C., Contell, J., Bori, L., Sancho, M., García-Molina, A., Valverde, A., & Segarvall, J. (2017b). Sperm kinematic, head morphometric and kinetic-morphometric subpopulations in the blue fox (*Alopex lagopus*). *Asian Journal of Andrology*, *19*(2), 154-159. https://doi.org/10.4103/1008-682X.188445

Soler, C., Cooper, T., Valverde, A., & Yániz, J. (2016). Afterword to Sperm morphometrics today and tomorrow special issue in Asian Journal of Andrology. *Asian Journal of Andrology*, *18*(6), 895-897. https://doi.org/10.4103/1008-682X.188451

Soler, C., Gadea, B., Soler, A., Fernández-Santos, M., Esteso, M., Núñez, J., Moreira, P., Núñez, M., Gutiérrez, R., Sancho, M., & Garde, J. (2005). Comparison of three different staining methods for the assessment of epididymal red deer sperm morphometry by computerized analysis with ISAS®. *Theriogenology*, *64*(5), 1236-1243. https://doi.org/10.1016/j.theriogenology.2005.02.018

Soler, C., García-Molina, A., Contell, J., Silvestre, M. A., & Sancho, M. (2015). The Trumorph® system: The new univ the morphology of living sperm. *Animal Reproduction Science*, *158*, 1-10. https://doi.org/10.1016/J.ANIREPROSCI.2015.04.001

Soler, C., Valverde, A., Bompart, D., Fereidounfar, S., Sancho, M., Yániz, J., Garcia-Molina, A., & Korneenko-Zhilyaev, Yu. A. (2017c). New methods of semen analysis by CASA. *Sel'skokhozyaistvennaya Biologiya*, *52*(2), 232-241. https://doi.org/10.15389/agrobiology.2017.2.232eng

Spencer, N. H. (2013). *Essentials of multivariate data analysis*. CRC Press. https://doi.org/10.1201/b16344

Taylor, JeremyF., Schnabel, RobertD., & Sutovsky, P. (2018). Review: Genomics of bull fertility. *animal*, *12*, s172-s183. https://doi.org/10.1017/S1751731118000599

Terán, E., Azcona, F., Ramón, M., Molina, A., Dorado, J., Hidalgo, M., Ross, P., Goszczynski, D., & Demyda-Peyrás, S. (2021). Sperm morphometry is affected by increased inbreeding in the Retinta cattle breed: A molecular approach. *Molecular Reproduction and Development*, *88*(6), 416-426. https://doi.org/10.1002/mrd.23475

Thurston, L. M., Siggins, K., Mileham, A. J., Watson, P. F., & Holt, W. V. (2002). Identification of Amplified Restriction Fragment Length Polymorphism Markers Linked to Genes Controlling Boar Sperm Viability Following Cryopreservation1. *Biology of Reproduction*, *66*(3), 545-554. https://doi.org/10.1095/biolreprod66.3.545

Toner, J. P., Mossad, H., Grow, D. R., Morshedi, M., Swanson, R. J., & Oehninger, S. (1995). Value of sperm morphology assessed by strict criteria for prediction of the outcome of artificial (intrauterine) insemination. *Andrologia*, *27*(3), 143-148. https://doi.org/10.1111/J.1439-0272.1995.TB01085.X

Valverde, A., Arenán, H., Sancho, M., Contell, J., Yániz, J., Fernández, A., & Soler, C. (2016). Morphometry and subpopulation structure of Holstein bull spermatozoa: variations in ejaculates and cryopreservation straws. *Asian Journal of Andrology*, *18*(6), 851. https://doi.org/10.4103/1008-682X.187579

Valverde, A., Madrigal-Valverde, M., Castro-Morales, O., Gadea-Rivas, A., Johnston, S., & Soler, C. (2019a). Kinematic and head morphometric characterisation of spermatozoa from the Brown Caiman (*Caiman crocodilus fuscus*). *Animal Reproduction Science*, *207*, 9-20. https://doi.org/10.1016/J.ANIREPROSCI.2019.06.011

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E-ISSN: 2215-3470
CC: BY-NC-ND

Valverde Abarca, A., Castro-Morales, O., & Madrigal-Valverde, M. (2019b). Sperm kinematics and morphometric subpopulations analysis with CASA systems: a review. *Revista de Biología Tropical*, *67*(6), 1473-1487. https://doi.org/10.15517/rbt.v67i6.35151

Vicente-Fiel, S., Palacín, I., Santolaria, P., Hidalgo, C. O., Silvestre, M. A., Arrebola, F., & Yániz, J. L. (2013). A comparative study of the sperm nuclear morphometry in cattle, goat, sheep, and pigs using a new computer-assisted method (CASMA-F). *Theriogenology*, *79*(3), 436-442. https://doi.org/10.1016/J.THERIOGENOLOGY.2012.10.015

Villaverde-Morcillo, S., Soler, A. J., Esteso, M. C., Castaño, C., Miñano-Berna, A., Gonzalez, F., & Santiago-Moreno, J. (2017). Immature and mature sperm morphometry in fresh and frozen-thawed falcon ejaculates. *Theriogenology*, *98*, 94-100. https://doi.org/10.1016/j.theriogenology.2017.04.051

Víquez, L., Barquero, V., Soler, C., Roldan, E. R. S., & Valverde, A. (2020). Kinematic Sub-Populations in Bull Spermatozoa: A Comparison of Classical and Bayesian Approaches. *Biology*, *9*(6), 138. https://doi.org/10.3390/biology9060138

Waberski, D., Suarez, S. S., & Henning, H. (2021). Assessment of sperm motility in livestock: Perspectives based on sperm swimming conditions *in vivo*. *Animal Reproduction Science*, *246,* 106849. https://doi.org/10.1016/j.anireprosci.2021.106849

Yániz, J. L., Palacín, I., Caycho, K. S., Soler, C., Silvestre, M. A., & Santolaria, P. (2018). Determining the relationship between bull sperm kinematic subpopulations and fluorescence groups using an integrated sperm quality analysis technique. *Reproduction. Fertilility and Development*, *30*(6), 919-923. https://doi.org/10.1071/rd17441

Yániz, J. L., Soler, C., & Santolaria, P. (2015). Computer assisted sperm morphometry in mammals: A review. *Animal Reproduction Science*, *156*, 1-12. https://doi.org/10.1016/J.ANIREPROSCI.2015.03.002

Yániz, J., Vicente-Fiel, S., Soler, C., Recreo, P., Carretero, T., Bono, A., Berné, J., & Santolaria, P. (2016). Comparison of different statistical approaches to evaluate morphometric sperm subpopulations in men. *Asian Journal of Andrology*, *18*(6), 819-823. https://doi.org/10.4103/1008-682X.186872

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