

The Effect of Sperm Concentration in the Ejaculate on Morphological Traits of Bull Spermatozoa

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Experiments were performed on 75 ejaculates obtained from 19 bulls representing different cattle breeds used at the Masovian Centre for Animal Breeding and Reproduction in Łowicz. Fresh ejaculates were measured in respect to their volume and sperm count in the ejaculates was determined. The ejaculates were classified based on the criterion of sperm concentration and divided into five groups. Sperm morphometric measurements were taken from each bull and assessment of semen morphology was done on the basis of examination under a microscope using preparations made from fresh ejaculates. For each slide, morphometric measurements were taken of 15 randomly selected spermatozoa characterised by normal morphology and well visible under the microscope. Additionally, in each preparation morphometry of 500 spermatozoa was evaluated, numbers of spermatozoa with normal morphology and morphological abnormalities were recorded and these were categorized into spermatozoa with major and minor defects. An insignificant correlation was observed between the sperm concentration in the ejaculate and morphological traits, dimensions and shapes of bull spermatozoa. The less concentrated ejaculates contained spermatozoa with a slightly larger head circumference and a more elongated head shape in comparison with the spermatozoa in the more concentrated ejaculates. The highest frequency of morphologically malformed spermatozoa, both in the case of primary and secondary alterations, was observed in ejaculates with sperm concentration of no more than $1000 \times 10^3/\text{mm}^3$.

Key words: Bull, ejaculate, sperm concentration, morphometric characteristics, spermatozoa.

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The insemination capability of spermatozoa is determined by their ultrastructure. Spermatozoa differ in shapes and sizes, and these parameters affect male fertility (SAILER *et al.* 1996; SUKCHAROEN *et al.* 1998; BRITO 2007; MORATO-MORALES *et al.* 2010). Many authors point out differences in the fertility of insemination bulls (EID *et al.* 1994; OSTERMEIER *et al.* 2001), although the causes for this are not entirely known. Undoubtedly, a high number of spermatozoa in a cubic content unit increases the efficacy of insemination. An important role may also be played by morphological and morphometric characteristics of the sex cells. The correct morphological structure of the sperm is a factor that guarantees successful arrival of spermatozoa at the oocyte and the activation of the egg cell. However, it does not ensure the continuity of embryonic development and gestation. It has been verified that the presence of spermatozoa with certain head modifications in the semen may result in

a lower quality of the embryo (DE JARNETTE *et al.* 1992) and miscarriage at the initial stage of pregnancy (CHENOWETH 2005). Perhaps the shape and size of male sex cells, and thus their motoric parameters determining their insemination potential, depend on the concentration of spermatozoa in the ejaculate. Some authors suggest a correlation between the morphometric characteristics of spermatozoa and sperm concentration in the ejaculates of dogs (RIJSSELAERE *et al.* 2004), stallions (DAVIS *et al.* 1993) and boars (KONDRACKI *et al.* 2006; BANASZEWSKA *et al.* 2009; KONDRACKI *et al.* 2011). The effect of the sperm count in boar ejaculates on the size and shape of spermatozoa was also confirmed (WYSOKIŃSKA *et al.* 2009). The present study is an attempt to analyse the interrelation between morphological parameters of spermatozoa and sperm concentration in bull ejaculates.

Material and Methods

The experiments were performed on 75 ejaculates obtained from 19 bulls representing different cattle breeds (Holstein-Frisian, Simentale, Limousine, Piemontese and Charolaise) used at the Masovian Centre for Animal Breeding and Reproduction in Łowicz. The bulls were kept and used for insemination in identical environmental conditions (buildings, feed administration and veterinary care). The ejaculates were collected using an artificial sheath. The fresh ejaculates were measured in respect of their volume based on a reading from a calibrated container in which the semen was collected. Additionally, sperm count in the ejaculates was determined. The sperm concentration was measured using the photometric method. The ejaculates were classified based on the criterion of sperm concentration and divided into five groups: ejaculates with a sperm concentration lower than or equal to $1000 \times 10^3/\text{mm}^3$ (group I), ejaculates with a sperm concentration of $1001\text{-}1300 \times 10^3/\text{mm}^3$ (group II), ejaculates with a sperm concentration of $1301\text{-}1600 \times 10^3/\text{mm}^3$ (group III), ejaculates with a sperm concentration of $1601\text{-}1900 \times 10^3/\text{mm}^3$ (group IV) and ejaculates with a sperm concentration higher than or equal to $1901 \times 10^3/\text{mm}^3$ (group V). Sperm morphometric measurements were taken from each bull and an assessment of semen morphology was done on the basis of examination under a microscope of preparations made from fresh ejaculates. The method of slide preparation had been described in a previous study (KONDRACKI *et al.* 2006). The preparations were examined under a Nikon E-50i light microscope using a 100x immersion lens. For each slide, morphometric measurements were taken of 15 randomly selected spermatozoa characterised by normal morphology and well visible under the microscope. The measurements were done by means of computer image analysis software (Screen Measurement v. 4.1, Laboratory Imaging S.r.o. LIM Czech Republic, Praha) following methodology prepared by KONDRACKI *et al.* (2005). A total of 1 125 measurements were taken including: sperm head perimeter, head area, sperm head length, sperm head width, flagellum length and overall sperm length. The following parameters of sperm morphology were calculated on the basis of the results of the morphometric measurements: sperm head width/length ratio, head length/overall sperm length ratio, sperm head length/flagellum length ratio, flagellum length/overall sperm length ratio, sperm head perimeter/overall sperm length ratio, sperm head area/overall sperm length ratio and the product of sperm head length and width/overall sperm length ratio. Additionally, in each preparation the morphology of 500 spermatozoa was evaluated, numbers of spermatozoa with normal morphology and

morphological abnormalities were recorded and these were categorized into spermatozoa with major and minor defects following the Blom classification system (BLOM 1981).

The results were statistically processed using analysis of variance according to the following mathematical model:

$$Y_{ij} = \mu + a_i + e_{ij}$$

where: Y_{ij} – trait value, μ – population mean, a_i – sperm concentration effect, e_{ij} – error.

Differences between means were tested using Tukey's test at $P \leq 0.05$ and $P \leq 0.01$.

Results

The data in Figure 1 show that the volume of the ejaculates was inversely proportional to the sperm concentration of the ejaculate. Ejaculates with lower sperm concentrations had greater volumes. Ejaculates with the sperm concentrations lower than or equal to $1000 \times 10^3/\text{mm}^3$ had the highest volume. The volume of those ejaculates was almost 2ml higher than the most densely concentrated ones (group V) ($P \leq 0.05$). The data reveal that as the sperm concentration rises, the ejaculate volume decreases. However, the dynamics of the observed changes is not stable. The volume variability of the ejaculates with sperm counts of ≤ 1000 to $1600 \times 10^3/\text{mm}^3$ (groups I, II and III) is insignificant, while a decrease in ejaculate volume in proportion to the rise of sperm concentration from $\leq 1000 \times 10^3/\text{mm}^3$ to $1600 \times 10^3/\text{mm}^3$ is only 0.2 ml and within the tolerance of the statistical error ($P \leq 0.05$). Sperm concentration above $1600 \times 10^3/\text{mm}^3$ is associated with a radical decrease of ejaculate volume down to 5.41 ml in group IV and to 5.11 ml in group V ($P \leq 0.05$).

Table 1 shows the relevant data for the morphometric characteristics of the spermatozoa in relation to the sperm count in the ejaculate. The data show that sperm dimensions depend only to a small extent on sperm concentration in the ejaculate. In most cases, the differences between the groups were insignificant and unconfirmed by statistical calculations. However, certain differences were observed in the width and circumference of sperm heads. In semen with a sperm count of $1301\text{-}1600 \times 10^3/\text{mm}^3$ (group III), spermatozoa had narrower heads as compared to semen with a sperm count of $1601\text{-}1900 \times 10^3/\text{mm}^3$ ($P \leq 0.01$). Spermatozoa from group I possessed the greatest circumference of the head, but the lowest sperm concentration. Circumferences in group I were

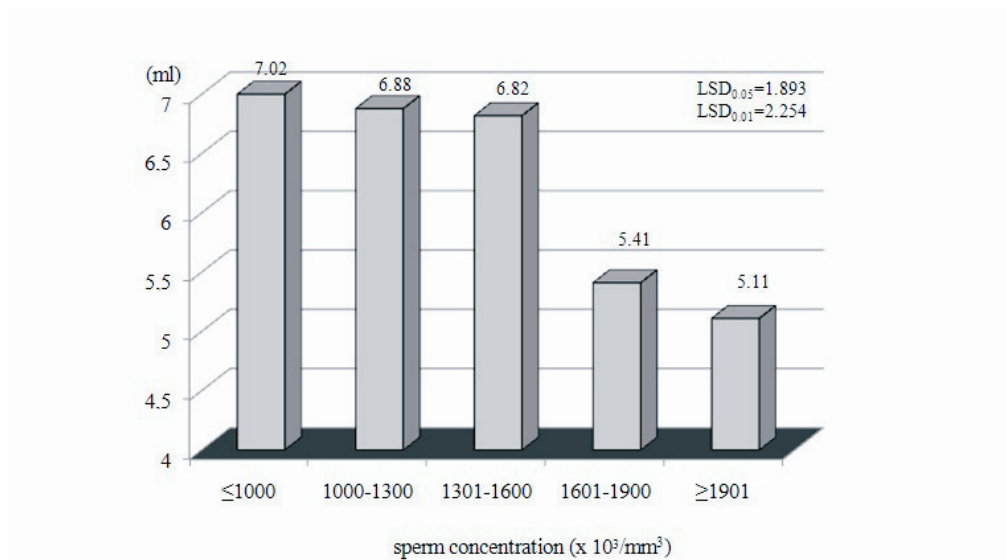


Fig. 1. Volume of ejaculate as a function of sperm concentration in bull ejaculate.

Table 1

Morphometric traits of sperms and sperm concentration in bull ejaculate

Item	Sperm concentration (x 10 ³ /mm ³)				
	Group I ≤1000	Group II 1000-1300	Group III 1301-1600	Group IV 1601-1900	Group V ≥1901
Number of ejaculates	16	17	15	14	13
Sperm concentration (x 10 ³ /mm ³)	710.94 ^A ± 195.66	1140.65 ^B ± 90.18	1464.73 ^C ± 88.43	1720.36 ^D ± 101.0	2123.77 ^E ± 327.22
Sperm head length (μm)	9.65 ^a ± 0.25	9.71 ^a ± 0.22	9.66 ^a ± 0.21	9.60 ^a ± 0.26	9.58 ^a ± 0.32
Sperm head width (m)	4.50 ^{acAB} ± 0.15	4.53 ^{abcAB} ± 0.13	4.47 ^{acA} ± 0.12	4.65 ^{bb} ± 0.15	4.50 ^{acAB} ± 0.24
Sperm head perimeter (m)	25.51 ^A ± 0.43	24.68 ^B ± 0.51	24.73 ^B ± 0.53	24.41 ^B ± 0.51	24.50 ^B ± 0.53
Sperm head area (m ²)	41.70 ^a ± 1.31	42.22 ^a ± 1.17	42.04 ^a ± 0.93	41.86 ^a ± 1.12	41.62 ^a ± 1.07
Sperm tail length (m)	61.34 ^a ± 0.98	61.29 ^a ± 1.02	61.61 ^a ± 0.71	61.12 ^a ± 0.95	61.42 ^a ± 0.57
Total sperm length (m)	70.99 ^a ± 1.06	71.01 ^a ± 1.08	71.27 ^a ± 0.76	70.79 ^a ± 1.17	70.99 ^a ± 0.71

Different superscripts mean significant differences among means within particular rows;
lower-case letters: P≤0.05, upper-case letters: P≤0.01.

larger by 0.78-1.10 μm than sperm heads in the other groups with higher sperm counts (P≤0.01).

Data concerning sperm shapes in the ejaculates with different sperm counts are presented in Table 2. Spermatozoa from the less concentrated ejaculates (groups I, II and III) had more elongate heads in comparison to spermatozoa in ejaculates with higher sperm counts, especially in relation to group IV ejaculates in which the differences turned out to be significant (P≤0.05). This is visible in the lower sperm head width to length ratio. A slightly different shape of spermatozoa from

groups III and V is evident in the smaller sperm head width to length ratio in proportion to the total length of the spermatozoon in comparison with group IV (P≤0.05) and groups I and II.

The data in Figures 2, 3 and 4 show a correlation between the sperm concentration in the ejaculate and the frequency of morphological alterations of the spermatozoa. Semen in group III, with sperm concentrations of 1301-1600x 10³/mm³ in the ejaculate (Fig. 2), was of the best quality. Over 97.3% of the spermatozoa had the correct morphological structure. Moreover, it contained the low-

Table 2

Indices of sperm morphology and sperm concentration in bull ejaculate

Item	Sperm concentration ($\times 10^3/\text{mm}^3$)				
	Group I ≤ 1000	Group II 1000-1300	Group III 1301-1600	Group IV 1601-1900	Group V ≥ 1901
Number of ejaculates	16	17	15	14	13
Sperm concentration ($\times 10^3/\text{mm}^3$)	710.94 ^A \pm 195.66	1140.65 ^B \pm 90.8	1464.73 ^C \pm 88.43	1720.36 ^D \pm 101.03	2123.77 ^E \pm 327.22
Width to length ratio of sperm head	46.61 ^{aAB} \pm 1.57	46.68 ^{aAB} \pm 1.41	46.25 ^{aA} \pm 1.49	48.49 ^{bb} \pm 2.03	47.06 ^{abAB} \pm 3.35
Ratio of head length to total sperm length	13.60 ^a \pm 0.33	13.68 ^a \pm 0.31	13.55 ^a \pm 0.27	13.56 ^a \pm 0.22	13.49 ^a \pm 0.38
Ratio of head length to sperm tail length	15.74 ^a \pm 0.44	15.85 ^a \pm 0.41	15.68 ^a \pm 0.36	15.70 ^a \pm 0.28	15.59 ^a \pm 0.51
Ratio of tail length to total sperm length	86.40 ^a \pm 0.32	86.32 ^a \pm 0.31	86.46 ^a \pm 0.27	86.34 ^a \pm 0.37	86.51 ^a \pm 0.38
Ratio of sperm head perimeter to total sperm length	34.52 ^a \pm 0.54	34.75 ^a \pm 0.75	34.70 ^a \pm 0.54	34.48 ^a \pm 0.43	34.51 ^a \pm 0.56
Ratio of sperm head area to total sperm length	58.74 ^a \pm 1.93	59.47 ^a \pm 1.82	58.99 ^a \pm 1.05	59.14 ^a \pm 1.46	58.62 ^a \pm 1.35
Ratio of sperm head length and width to total sperm length	61.19 ^{ab} \pm 2.73	62.03 ^{ab} \pm 2.41	60.53 ^a \pm 2.07	63.07 ^b \pm 2.29	60.68 ^a \pm 3.47

Different superscripts reflect significant differences among means within particular rows; lower-case letters: $P \leq 0.05$, upper-case letters: $P \leq 0.01$.

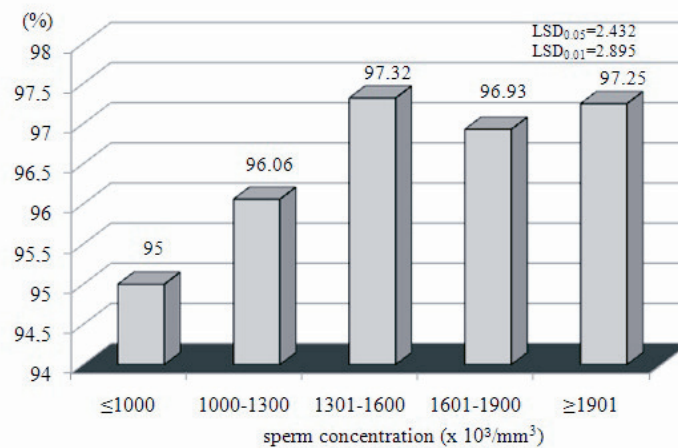


Fig. 2. Normal spermatozoa as a function of sperm concentration in bull ejaculate.

est number of spermatozoa with primary changes (Fig. 3), only 0.24%, i.e. 0.82% less than in the ejaculates in group I, having the lowest sperm count ($P \leq 0.05$). Group I ejaculates were found to contain the most spermatozoa with secondary morphological changes (Fig. 4). The frequency of these changes was 3.94%, over 2% higher than in the ejaculates with the highest sperm concentration ($P \leq 0.05$). Group I also contained the most spermatozoa with primary changes.

Discussion

The presented data suggest a correlation between the morphological traits of spermatozoa and sperm concentration in the ejaculate. This is caused by differences between the sizes and shapes of the spermatozoa and the frequency of incidence of morphological alterations of spermatozoa in the ejaculate groups that differed in the sperm count. In ejaculates with the lowest sperm

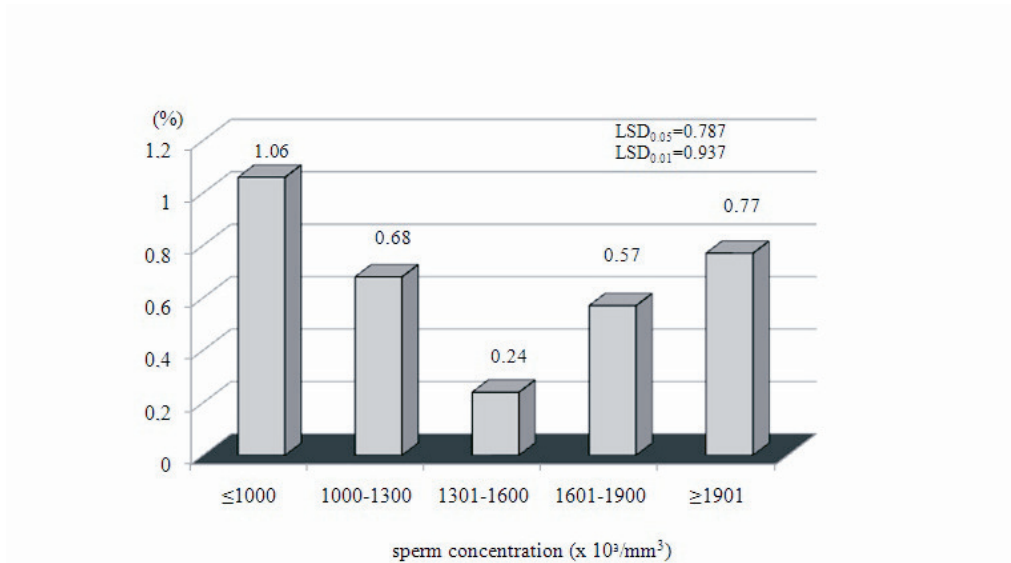


Fig. 3. Sperm with major abnormalities as a function of sperm concentration in bull ejaculate.

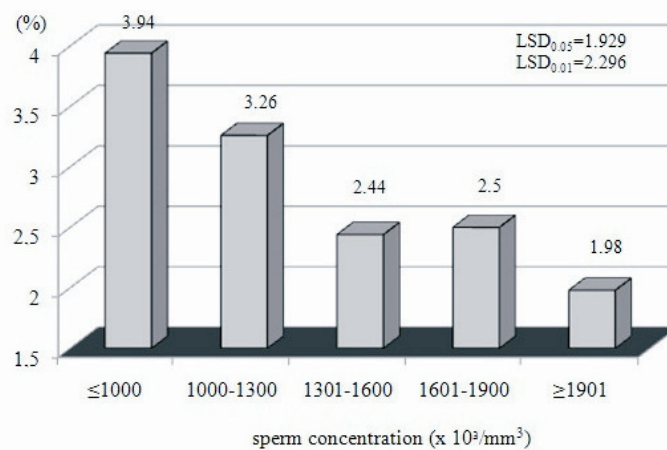


Fig. 4. Sperm with minor abnormalities as a function of sperm concentration in bull ejaculate.

concentrations, spermatozoa had a larger head circumference as compared with spermatozoa from the more concentrated ejaculates. Similar observations were made in studies performed on boar for which the less concentrated ejaculates contained spermatozoa with greater head dimensions (BANASZEWSKA *et al.* 2009; KONDRACKI *et al.* 2011). Variable sperm head dimensions may result from different chromatin structure. Support for this hypothesis can be found in a study showing that sperm head dimensions depend on chromatin structure in bulls (SAILER *et al.* 1996). A mammalian sperm head almost entirely consists of chromatin. Therefore, its shape is determined by DNA organisation (STEINHOLT *et al.* 1994). BELETTI *et al.* (2005) showed that spermatozoa with morphologically well-formed heads may be dysfunctional

as regards chromatin condensation, reflected in smaller dimensions of the sperm head. Some authors think that even subtle modifications of the sperm head shape may be associated with changes in chromatin structure in the nucleus (HINGEST *et al.* 1995; SAILER *et al.* 1996; OSTERMEIER *et al.* 2001) which may lead to reduced fertility (EVENSON & WIXON 2006). The results of the present study show that in ejaculates with a sperm concentration of 1301-1600x 10³/mm³ (group III) the spermatozoa have narrower heads as compared to sperms in the more concentrated ejaculates. Moreover, the spermatozoa from ejaculates with a concentration below 1000x 10³/mm³ (group I, II and III) had a more elongated shape in comparison with spermatozoa in the more concentrated ejaculates. OSTERMEIER *et al.* (2001) identified spermatozoa

with narrower and more elongate heads in the semen of highly fertile bulls as compared to the ejaculates of less fertile ones. The progressive mobility of a sperm is an indicator of male fertility. Sperm mobility can also be affected by head size. According to MALO *et al.* (2006), spermatozoa with more elongate heads move faster than those with round heads. Some authors point out differences in the intensity and style of movement of spermatozoa depending on the shape of their heads (THURSTON *et al.* 2001). Spermatozoa with greater ellipticity (head length/head width) and broader midpieces are less capable of progressive rectilinear movement (GIL *et al.* 2009). Many authors have identified a correlation between the midpiece length and tail length and sperm mobility (GOMENDIO & ROLDAN 1991; ANDERSON & DIXON 2002; MALO *et al.* 2006; BIERŁA *et al.* 2007; LÜPOLD *et al.* 2009; CIFTCI & ZÜLKADIR 2010). In ejaculates with a high percentage of progressively moving spermatozoa, the sperms have longer tails as compared to ejaculates containing less mobile spermatozoa (NOORAFSHAN & KARBALAY-DOUST 2010). In boar, spermatozoa with the longest tails were present in dilute ejaculates (KONDRACKI *et al.* 2011). An inverse correlation was observed in a study of dog semen (RIJSSELAERE *et al.* 2004). In dog ejaculates with a lower sperm concentration, the spermatozoa had shorter tails as compared with the spermatozoa in the more concentrated ejaculates. Additionally, the less concentrated ejaculates contained spermatozoa with shorter and narrower heads with a smaller circumference and smaller surface area (RIJSSELAERE *et al.* 2004). Slightly different observations were made in a study of stallion semen – the more concentrated ejaculates were found to contain spermatozoa with smaller and less elongated heads as opposed to the less concentrated ejaculates (DAVIS *et al.* 1993). Sperm dimensions can also be affected by the semen storage and preservation method (ARRUDA *et al.* 2002; RUBIO-GUILLÉN *et al.* 2007; HIDALGO *et al.* 2008). A study found that fresh bull ejaculates contained spermatozoa with smaller heads in comparison with diluted ejaculates (GRAVANCE *et al.* 1998).

Data reported herein show that the ejaculates with sperm concentrations of 1301-1600x 10³/mm³ (group III) had the highest frequency of morphologically well-formed spermatozoa. The morphological structure of semen can be conditioned by many factors. The frequency of morphological deviations is affected by genetic predisposition (CHENOWETH 2005), seasonal and environmental factors, as well as individual predisposition (DAHLBOM *et al.* 1997; BOERSMA *et al.* 1999; PURWANTARA *et al.* 2010). Certain studies have identified considerable differences in the fre-

quency of morphological deviations of spermatozoa in the semen of variously aged breeders (SÖDERQUIST *et al.* 1996; PADRIK & JAAKMA 2002; HALLAP *et al.* 2005; MAKHZOOMI *et al.* 2008; SARDER 2008). Some studies suggest that less concentrated ejaculates contain fewer spermatozoa with morphological alterations, while as the sperm concentration rises, the percentage of morphological anomalies also increases (RIJSSELAERE *et al.* 2004). Higher sperm concentration in the ejaculate normally entails higher sperm concentration in the outlet tract, where the spermatozoa are stored and undergo changes that condition their fertilisation capability. High sperm concentration in the outlet tract may deteriorate the environment for spermatozoan development and lead to a higher frequency of morphologically impaired cells with altered dimensions and shapes. The results of the present study do not support this thesis because the least concentrated ejaculates exhibited the highest frequency of morphological alterations of the sperms.

An insignificant correlation was observed between the sperm concentration in the ejaculate and the morphological traits, dimensions and shapes of bull spermatozoa. The less concentrated ejaculates contained spermatozoa with a slightly larger head circumference and a more elongate head shape in comparison with the spermatozoa in the more concentrated ejaculates. The highest frequency of morphologically malformed spermatozoa, both in the case of primary and secondary alterations, was observed in ejaculates with a sperm concentration of no more than 1000 x 10³/mm³.

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