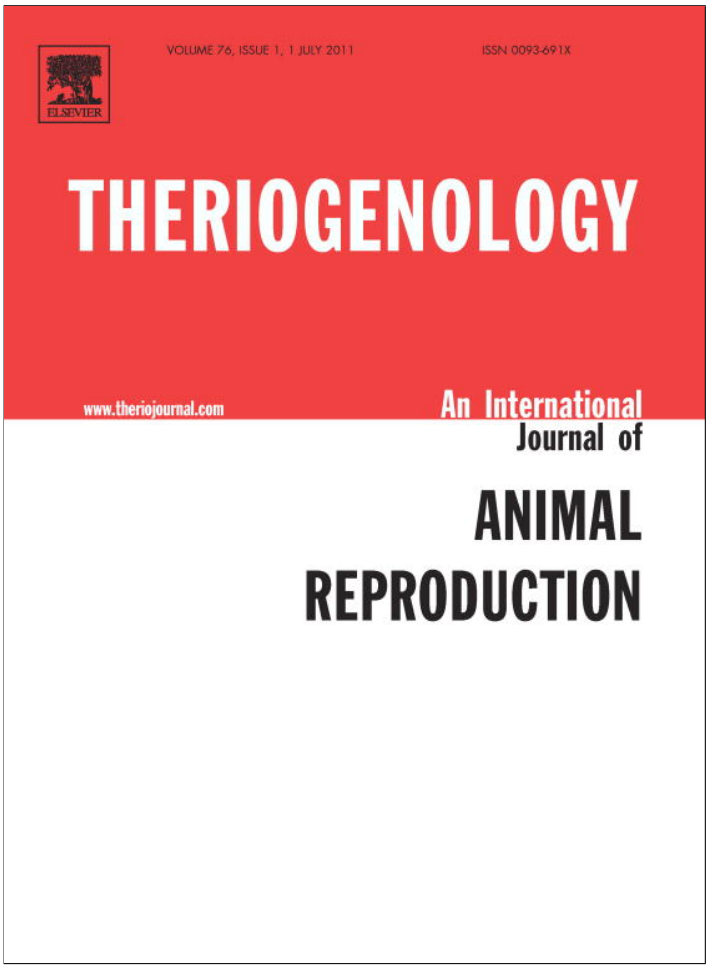

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Major morphological sperm abnormalities in the bull are related to sperm DNA damage

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Abstract

The influence of sperm morphology and chromatin integrity on bull fertility suggests a strong but undefined biological relationship between these two parameters. In this study we explore this relationship, making use of the Sperm Chromatin Dispersion test, which allows simultaneous observation of sperm abnormalities and DNA fragmentation. Based on spermatozoa from 17 Holstein-Friesian bulls, we determined a relationship between DNA fragmentation and the presence of the "so called" *major-type* sperm defects. Values for DNA fragmentation index (mean ± SEM) calculated from cells with *major-type* abnormalities were significantly ($P < 0.05$) higher (85.05 ± 5.00%) than those from abnormal forms classified as *minor-type* (17.89 ± 5.55%). Some of the sperm abnormalities, such as double forms, narrow base heads, small heads, shortened tails and proximal cytoplasmic droplets, were only associated with sperm showing fragmented DNA. The simultaneous assessment of sperm morphology and DNA fragmentation has the potential to improve the efficacy of sperm quality assessment in this species.

Keywords: Sperm DNA fragmentation; Sperm morphology; Major abnormalities; Minor abnormalities; Sperm Chromatin Dispersion

1. Introduction

Sperm abnormalities have long been associated with male infertility and sterility [1–3] such that their assessment is a fundamental component of the analysis of semen quality [4]. Sperm structure and morphology appear to have a major impact on the success rate of fertilization, early embryonic development and pregnancy rate in artificial reproduction practice [5–8]. Recent studies have also indicated the importance and contribution of DNA and/or chromatin status in the assessment of fertility [9–12]. Nevertheless, the application of this chromatin analysis is still a relatively new concept for the animal production industries and is currently not incorporated as standard practice.

Given that spermatogenesis and sperm maturation is a highly dynamic process in terms of sperm DNA replication and packaging, it is probable that any heritable or environmental perturbation to this process will be reflected in the production of a morphologically abnormal spermatozoa; some of these ejaculates will undoubtedly contain spermatozoa with defective chromatin structure or an abnormally high level of DNA damage, so that one might predict a close relationship between sperm morphology and DNA quality.

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Demonstration of a direct association between abnormal sperm morphology and a high level of DNA fragmentation has not always been obvious and in some instances this relationship has been controversial. While there have been several studies supporting the hypothesis that abnormal sperm morphology is statistically associated with an increase in the incidence of chromosomal abnormalities [13,14], chromatin instability [3] or abnormal chromatin structure [15], there are others where this conclusion is not supported [16].

The analysis of morphological sperm abnormalities in the bull ejaculate is a common veterinary practice prior to the sale of the bull, natural service and storage of frozen semen [3]. There are currently 25 recognised different variants of abnormal bovine sperm morphology. However, when reporting bovine sperm abnormalities, it has been convention to recognise two populations of sperm in what Blom denotes as *major-type* and *minor-type* abnormalities [17] (see Table 1). This classification system is based on the relative importance of the sperm abnormality to fertility. Thus, while the major abnormalities have been correlated to impaired fertility [3], minor defects do not necessarily indicate a disturbance of spermatogenesis, but nevertheless, could cause a reduction in fertility if they are present in large proportions within the ejaculate [18].

The aim of the present study was to explore the relationship between the incidence of major and minor bovine abnormal sperm morphology and sperm DNA fragmentation. It is hypothesised that morphologically abnormal spermatozoa are likely to present DNA fragmentation.

Table 1
Major and minor sperm defects in the bull [17].

Major	Minor
Underdeveloped	Narrow heads
Double forms	Small normal heads
Acrosome defect (knobbed acrosome)*	Giant and short broad heads
Decapitated sperm defect (active tails)	Free normal heads
Diadem defect*	Detached acrosome membranes
Pear-shaped defect	Abaxial implantation
Narrow at base	Distal droplet
Abnormal contour	Simple bent tail
Small abnormal heads	Terminally coiled tail
Free pathological heads	Other cells
Corkscrew defect	
Tail stump*	
Proximal droplet	
Pseudodroplet	
Strongly coiled or folded tail ("Dag" defect)	
Broken neck	

* Abnormalities with a genetic origin.

2. Materials and methods

2.1. Sperm preparation

Frozen-thawed spermatozoa from 17 healthy sexually mature Holstein-Friesian bulls from an artificial insemination centre in Buenos Aires (Argentina) were evaluated. The ejaculates were obtained using an artificial vagina and rapidly diluted in TEST-yolk extender containing 7% (v/v) glycerol at 37 °C. The diluted semen was cooled at 5 °C for 2h and then equilibrated for an additional 2h at 5 °C prior to freezing. Sperm samples were frozen in 5 ml polyvinyl straws (IMV Eagle, France) placed 4 cm above liquid nitrogen surface for 10 min after which time they were plunged directly into liquid nitrogen and stored until thawing. For analysis, frozen straws were thawed in a water-bath at 37 °C for 30s. The semen was then maintained at 37 °C for no longer than 2 h until assessed for sperm morphology and DNA fragmentation.

2.2. Sperm DNA fragmentation assessment

The Sperm-*Bos*-Halomax ® kit (ChromaCell SL, Madrid, Spain) was used for the assessment of sperm DNA fragmentation. This methodology is based on the Sperm Chromatin Dispersion (SCD) test, the details of which have been described elsewhere [19,20]. Briefly, 25 μl of diluted spermatozoa (10×10^6 ml^{-1}) were added to a vial with low melting point agarose at 37 °C and mixed thoroughly. Approximately 15 μl of the sperm suspension was spread onto a pre-treated microgel slide provided in the Sperm-*Bos*-Halomax ® kit, covered with a coverslip and cooled at 4 °C for 5 min. The coverslip was then carefully removed, and the slide placed horizontally in 10ml of the lysing solution provided within the kit. Finally, the slides were washed in dH_2O for 5 min, dehydrated in a sequential series of ethanol baths (70, 90 and 100% v/v) and then air-dried. Slides were stained with two fluorochromes: mercuridibromofluoresceine (Panreac, Barcelona, Spain) for residual protein staining and propidium iodide (Sigma, St Louis, MO, USA) for DNA staining. Five hundred spermatozoa per sample were randomly scored using a 63× objective on a Leica DMLB fuorescence microscope (Leica Microsystems, Wetzlar, Germany) equipped with two independent green (I3) and red (Y3) filters. Digital images were produced as tiff images using a cooled Leica DCF 300 camera

mounted onto a Leica DM microscope with single-band pass filters (FITC-3540B-536/617; Cy5-4040A-492/516; Semrock, Rochester, NY).

2.3. In situ Nick translation (ISNT)

To validate the results of the SCD Test, ISNT of the DNA breaks was performed on sperm samples treated with the lysing agent provided in the SCD kit. After protein lysis of microgel-embedded spermatozoa, the slides were thoroughly washed 4 times in PBS (Sigma, St Louis, MO, USA) for 5 min each and then incubated for 5 min in an excess of reaction buffer for DNA-polymerase I (10 mM Tris–HCl, 5 mM MgCl2, 7.5 mM DTT, pH 7.5). Following this treatment, 100 ml of reaction buffer containing 25 units of DNA-polymerase I (New England BioLabs, Beverly, USA) and biotin-16-dUTP (Roche, Spain) in the nucleotide mix, was deposited onto the slide, covered with a plastic coverslip and incubated in a moist chamber for 25 min at 37 °C. After washing in TBE buffer (Sigma, St Louis, MO, USA), the slides were dehydrated in sequential series of ethanol baths (70, 90 and 100% v/v) and air-dried. The incorporated biotin-16-dUTP was detected by incubation with an appropriate antibody conjugated with FITC (Roche, Spain) for 30 min. Slide preparations were directly analyzed or counterstained with propidium iodide (2 μg/ml) in Vectashield (Vector, Burlingame, CA, USA) antifade mounting media and analysed; a minimum of 500 spermatozoa per sample were scored.

2.4. Morphological assessment of spermatozoa

Slides containing the semen samples were prepared by carefully placing a drop of the diluted sperm suspension across a clean microscopic slide heated to 37 °C. The slides were allowed to air dry for 2 h, followed by a treatment with a 96% (v/v) ethanol solution (Sigma, St Louis, MO, USA) for a further 5 min. After air-drying for 30 min, slides were re-hydrated in distilled water, and then stained with a 10% (v/v) aqueous solution of eosin (Sigma, St Louis, MO, USA) for 1 min. The stained slides were further washed in dH_2O, and then stained with aniline blue (Fischer Scientific, USA) for 3 to 5 min. Finally, the slides were gently rinsed with dH_2O for 2min to remove any excess debris and air-dried. Sperm morphology was also assessed on SCDt processed slides; this approach allowed DNA-fragmented sperm and abnormal morphology to be analysed simultaneously.

A number of classification systems exist for sperm abnormalities in the bull. In this study, sperm abnormalities were classified as *major* and *minor* sperm defects as defined by Blom [17,21] (see Table 1).

2.5. Index calculation and statistical analysis

Statistical analysis was performed using the SPSS v.14.0.

A non-parametric U Mann Whitney test was used to detect significant differences in the level of DNA fragmentation calculated by the SCDt and the ISNT assay.

A non-parametric U Mann Whitney test was also performed to detect differences in the incidence of sperm morphological abnormalities in spermatozoa with or without DNA fragmentation and in the incidence of sperm DNA fragmentation in spermatozoa with major and minor abnormalities.

As part of the analysis, various indexes of sperm DNA fragmentation and sperm abnormalities were calculated: *DNA fragmentation index* = percentage of DNA-fragmented sperm cells in (i) the total of spermatozoa (DFI), (ii) in the total of spermatozoa with major abnormalities (DFIm), (iii) in the total of spermatozoa with minor abnormalities (DFImn); *Abnormalities index* = percentage of morphologically abnormal sperm cells in (i) the total number of spermatozoa (AI), (ii) in the total of DNA-fragmented spermatozoa (AIF) and (iii) in the total of non DNA-fragmented spermatozoa (AINF); *minor abnormalities index* = percentage of morphologically minor abnormal sperm cells in (i) the total of spermatozoa (mnAI), (ii) in the total of DNA-fragmented spermatozoa (mnAIF) and (iii) in the total of non DNA-fragmented spermatozoa (mnAINF); *major abnormalities index* = percentage of morphologically major abnormal sperm cells in (i) the total of spermatozoa (mAI), (ii) in the total of DNA-fragmented spermatozoa (mAIF) and (iii) in the total of non DNA-fragmented spermatozoa (mAINF).

3. Results

3.1. Validation of the SCDt assay

Bull spermatozoa with damaged DNA and treated with the Sperm Chromatin Dispersion test (SCDt), gave rise to nucleoids with a central core and a large peripheral halo of dispersed DNA fragments, whereas those spermatozoa with undamaged DNA showed no halo of chromatin dispersion or the presence of very small and compact haloes surrounding a more compact nuclear core (Fig. 1A). The presence of DNA breaks in the dispersed haloes was validated by means of direct incorporation of labelled nucleo-

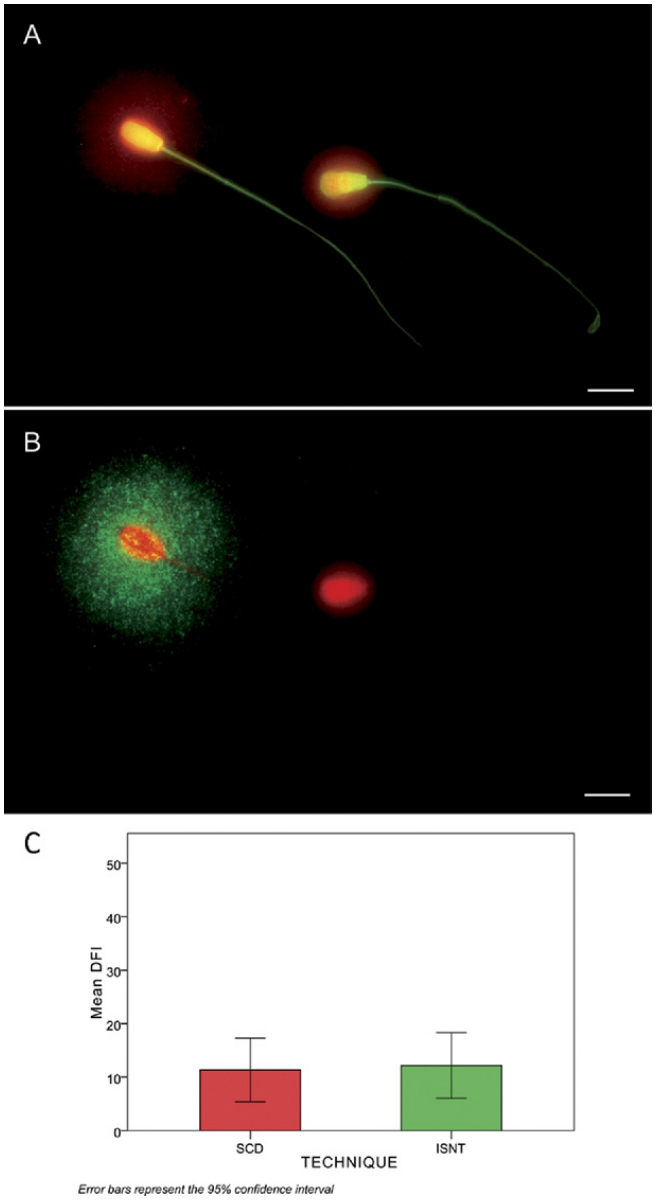

Fig. 1. Bull spermatozoa after the SCD test and the ISNT assay. (A) Sperm nuclei containing fragmented DNA release their DNA loops forming large halos of chromatin dispersion; sperm nuclei with intact DNA produce very small halos. (B) *In situ* nick translation assay on SCD processed bull spermatozoa. The assay detects the presence of DNA breaks by the incorporation of labelled nucleotides (green) using the DNA polymerase I. Propidium iodide (red) was used as a counterstain. Scale bar—5 μm. (C) Mean DNA fragmentation index (DFI) of the 17 semen samples analysed by SCDt and ISNT.

tides using an *in situ* polymerase reaction environment. In this assay system, only sperm cells displaying large haloes of chromatin dispersion showed evidence of being labelled (Fig. 1B). Thus, the presence or absence of DNA fragmentation can be directly assessed in the bull by examining the size of the halo. According to this criterion, the DFI of the 17 semen samples studied was calculated using both techniques, SCDt and ISNT. Results showed that the mean DFI calculated by the SCDt (mean ± standard error, SEM) (11.35% ± 2.81%) was similar to the one obtained by ISNT (12.18% ± 2.90%); no significant difference was found in the DFIs calculated by these techniques in any of the samples analysed (Fig. 1C).

3.2. The relationship between SCDt and sperm abnormalities

Scoring results of morphological abnormalities by eosin-aniline blue staining and SCDt are shown in Table 2. No statistical difference was found between both methods in the percentage of morphologically abnormal sperm cells in the total number of spermatozoa in any of the defects evaluated.

A benefit of using the SCD test to assess DNA fragmentation of morphologically abnormal sperm is that it allowed a simultaneous examination of morphology and DNA fragmentation. Four different combinations of sperm cells were detectable in the ejaculates of the 17 bulls examined with the SCDt: (a) morphologically normal sperm cells containing intact DNA (mean ± SEM) (40.96% ± 1.33%); (b) morphologically abnormal sperm cells with intact DNA (9.04% ± 1.33%); (c) morphologically normal sperm cells with damaged DNA (19.33% ± 2.50%);

Table 2
Percentage of morphologically abnormal sperm cells in the total number of spermatozoa found in the 17 samples analysed by both eosin-aniline blue staining and SCDt.

Morphological abnormalities	Eosin-aniline blue staining	SCDt
Major		
Double forms	1.40 ± 0.67	1.28 ± 0.56
Pear-shaped defect	3.12 ± 1.57	2.92 ± 1.06
Narrow at base	0.59 ± 0.95	1.52 ± 0.90
Small abnormal heads	3.86 ± 0.92	2.76 ± 0.72
Free pathological heads	9.20 ± 1.87	7.90 ± 1.87
Tail stump	2.50 ± 0.53	2.57 ± 0.75
Whip tail	1.41 ± 0.47	2.14 ± 0.94
Proximal droplet	1.98 ± 0.84	1.19 ± 0.64
"Dag" defect	3.00 ± 0.78	2.10 ± 0.98
Total Major	8.07 ± 0.59	7.07 ± 0.98
Minor		
Giant and short broad heads	2.07 ± 0.79	1.47 ± 0.75
Simple bent tail	1.00 ± 0.49	1.15 ± 0.54
Terminally coiled tail	10.45 ± 1.90	9.45 ± 1.40
Total Minor	18.90 ± 2.68	16.90 ± 2.26
Total Major + Minor	21.93 ± 1.44	23.97 ± 2.40

Values are mean ± SEM.
*Significant differences are found between eosin-aniline blue staining and SCDt, $P < 0.05$, Mann Whitney U test.

and (d) morphologically abnormal sperm cells with damaged DNA (30.67% ± 2.50%).

Figures 2 and 3 show the range of the various types of morphological abnormalities found in bovine spermatozoa that displayed fragmented and non-fragmented DNA respectively. Proportions (mean ± SEM) of those abnormalities found in the samples analysed are shown in Table 3. Note that some of the abnormalities such as double forms, narrow base, small head, short tail and proximal droplet were only associated with spermatozoa showing fragmented DNA.

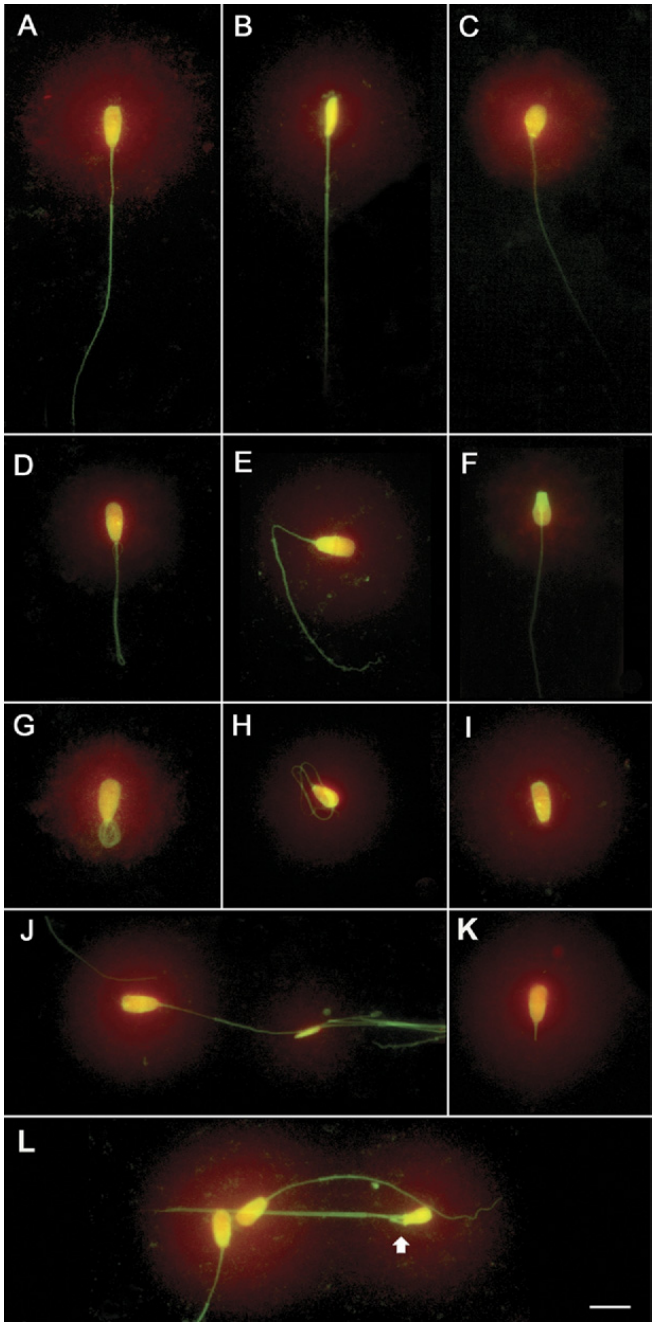

Fig. 2. Different morphological abnormalities found in DNA-fragmented bull sperm cells. (A) normal, (B) whip tail, (C) round head, (D) terminally coiled, (E) bent tail, (F) neck bent tail, (G) dag defect, (H) coiled tail, (I) decapited head, (J) small abnormal head, (K) short tail, (L) double form. Scale bar—5 μm.

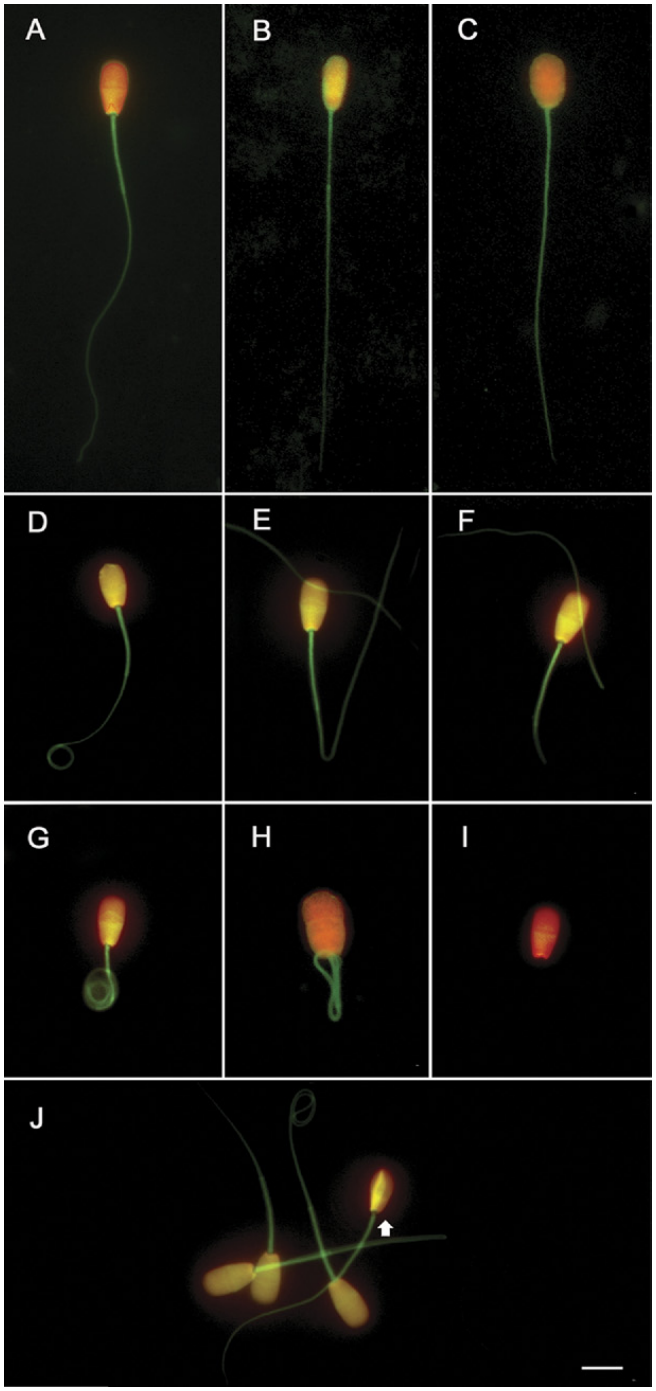

Fig. 3. Different morphological abnormalities found in non DNA-fragmented bull sperm cells. (A) normal, (B) whip tail, (C) round head, (D) terminally coiled, (E) bent tail, (F) broken tail, (G) dag defect, (H) giant head, (I) decapited head, (J) small abnormal head. Scale bar—5 μm.

The total mean (± SEM) proportion of bovine sperm abnormalities (AI) was significantly (ANOVA, $P < 0.05$)

Table 3
Major and minor abnormalities present in the DNA-fragmented and non DNA-fragmented categories found in the 17 samples analysed.

Morphological abnormalities	Non DNA-fragmented	DNA-fragmented
Major		
Double forms	0.00 ± 0.00*	2.55 ± 1.04*
Pear-shaped defect	0.08 ± 0.04*	5.77 ± 1.91*
Narrow at base	0.00 ± 0.00	3.03 ± 1.76
Small abnormal heads	0.00 ± 0.00*	5.52 ± 1.08*
Free pathological heads	0.33 ± 0.13*	15.47 ± 2.69*
Tail stump	0.00 ± 0.00*	5.15 ± 1.22*
Whip tail	0.02 ± 0.02	4.25 ± 1.76
Proximal droplet	0.00 ± 0.00	2.37 ± 1.23
"Dag" defect	0.66 ± 0.28	3.54 ± 1.90
Total Major	1.30 ± 0.39*	46.88 ± 4.93*
Minor		
Giant and short broad heads	0.23 ± 0.11	2.71 ± 1.45
Simple bent tail	0.58 ± 0.32	1.72 ± 1.03
Terminally coiled tail	13.02 ± 1.88*	5.87 ± 1.70*
Total Minor	16.78 ± 2.46	14.46 ± 3.22
Total Major + Minor	18.08 ± 2.66*	61.34 ± 5.00*

Values are mean ± SEM.
* Significant differences are found between DNA-fragmented and non DNA-fragmented sperm cells, $P < 0.05$, Mann Whitney U test.

higher in those sperm with fragmented DNA (61.34% ± 5.00%) compared to those with non-fragmented DNA (18.08% ± 2.66%). This was also the case when individual bulls where analysed separately (X^2 test, $P < 0.05$). Sperm morphological data of both groups (DNA-fragmented and non DNA-fragmented sperm cells) were then categorised into minor and major sperm abnormalities and were analysed separately. No significant differences were found in the mean (± SEM) proportion of minor sperm abnormalities between DNA-fragmented (14.46% ± 3.22%) and non DNA-fragmented (16.78% ± 2.46%) sperm cells. However, a similar analysis of major sperm abnormalities showed that the mean (± SEM) proportion of cells with fragmented DNA (46.88% ± 4.93%) was significantly higher (ANOVA, $P < 0.05$) than those with non-fragmented DNA (1.30 ± 0.39). This finding was also consistent when each sperm sample was analysed separately (X^2 test, $P < 0.05$) (Fig. 4).

In addition, values for DFI (mean ± SEM) calculated from spermatozoa with major abnormalities were significantly higher (U Mann Whitney test, $P < 0.05$: 85.05% ± 5.00%) than those from abnormal forms classified as minor (17.89% ± 5.55%). This finding was also consistent when each sperm sample was analysed separately (X^2 test, $P < 0.05$).

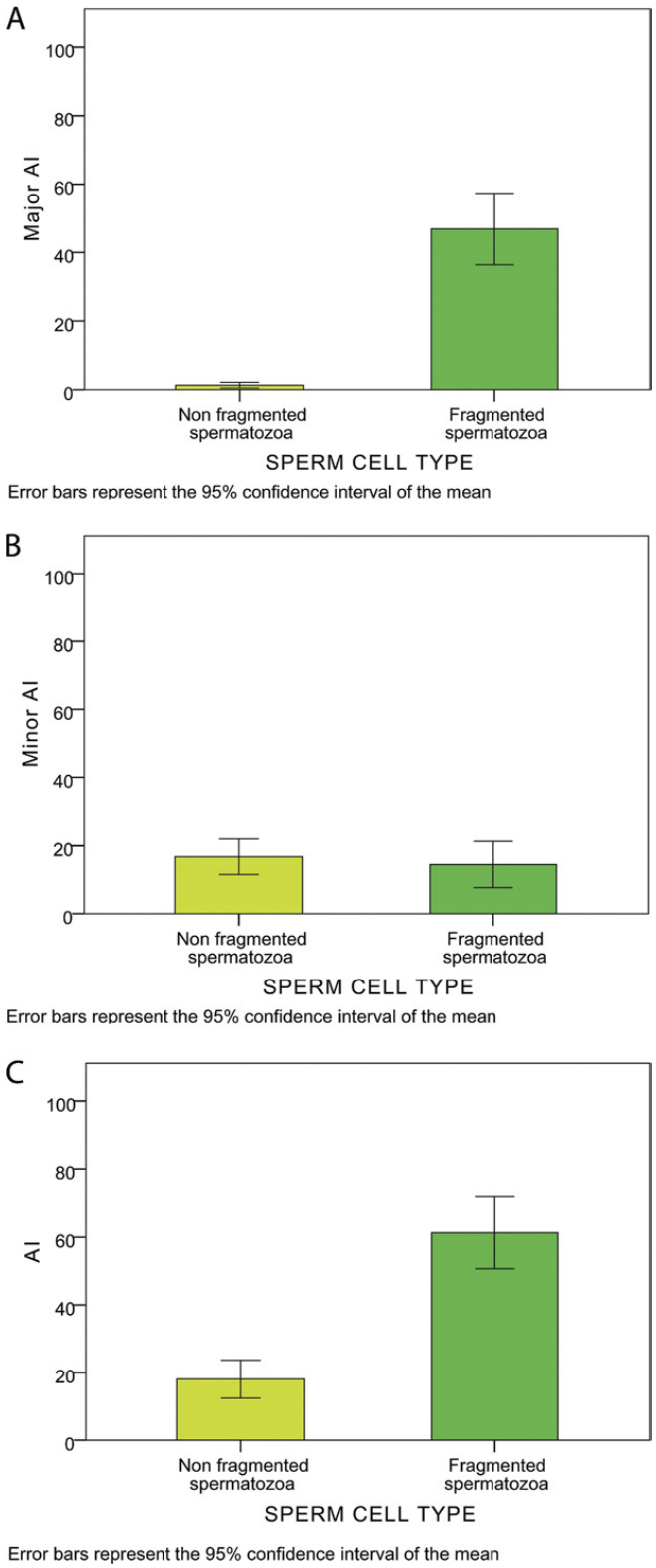

Fig. 4. (A) Mean morphological abnormalities index of sperm with fragmented DNA (AIF) and sperm with non-fragmented DNA (AINF). (B) Mean minor morphological abnormalities index determined from sperm with fragmented DNA (mnAIF) and sperm with non-fragmented DNA (mnAINF). (C) Mean major morphological abnormalities index determined from sperm with fragmented DNA (mAIF) and sperm with non-fragmented DNA (mAINF) cells (AI).

4. Discussion

This study showed a clear relationship between bull abnormal sperm morphology and sperm DNA fragmentation. In particular, the incidence of abnormalities classified as *major-type* showed higher values for DNA fragmentation index (DFI) than those abnormal forms classified as *minor-type*. The freeze-thaw process did not affect the relationship between DNA fragmentation and major sperm defects. As the DFIs of the sperm samples in this study were within the normal ranges established in the literature for reproductively sound bulls, it appears that no major effect in DNA integrity was associated with cryopreservation immediately following thawing. Several studies have shown that bull sperm chromatin structure remains unaltered after the cryopreservation process [22,23]. Sperm DNA resistance to cryopreservation has also been described in other animal species such as stallion, boar, human and koala [24–27].

During the last decade, several studies have explored the relationship between sperm shape and DNA integrity; however, the correlation of sperm morphology and other sperm parameters with DNA damage is still very controversial. Some studies have shown an association between sperm morphology and chromatin integrity or chromosomal abnormalities [15,28–30]; while others suggest that this association is not consistent [31–33]. Muriel et al reported a relationship between the presence of aneuploidy and DNA damage in human spermatozoa [34]. Other publications have described a poor correlation between the classical parameters of a routine semen analysis and the incidence of DNA damage [34,35]. There could be several reasons why these studies have not found a clear relationship between sperm morphology and DNA damage. Firstly, different morphological assessment criteria have been used in these studies [4,36]; unfortunately there still appears to be no universal acceptance on a homogeneous classification system for sperm morphology [4]. Secondly, sperm morphology and DNA damage are usually assessed in the same semen sample but not on the same sperm cell. Most of the tests available for detecting DNA damage do not allow the simultaneous evaluation of sperm morphology. The SCD test used in this study allows DNA damage to be assessed at the single cell level so that relationship between morphology and DNA damage can be directly observed. Thirdly, it is possible that sperm DNA damage, as reported in this study, is closely related to specific sperm types of abnormal morphology. In fact, the main finding of the present study was that major morphological abnormalities are primarily and, in some cases, exclusively, associated with DNA damaged spermatozoa while minor morphological abnormalities are present in both DNA-damaged and intact DNA spermatozoa.

But how did these morphologically abnormal sperm with fragmented DNA originate? Given the scarce information about the origin of DNA fragmentation in the sperm, a direct explanation of the relationship between DNA damage and abnormal morphology obviously needs to be speculative. Nevertheless, three main hypotheses have been proposed to explain the presence of sperm DNA fragmentation. In the first scenario, the exchange of histones by protamines in mid-spermiogenesis involves the participation of enzymes that cut and ligate the DNA to release the torsional stress produced in this process. Failure to repair these breaks because of an incomplete chromatin maturation process may result in morphological abnormalities [37,38]. The second hypothesis is consistent with the concourse of an apoptosis-related DNA scenario as it happens in somatic cells [39]. Congruent with this idea is the presence of activated caspases 8, 1 and 3 in the post-acrosomal region and of caspase 9 in the midpiece [40]. Moreover, recent evidence of the possible presence of an endogenous nuclease has been reported in human, hamster and mouse spermatozoa [41]. The third hypothesis proposes that DNA fragmentation is the consequence of oxidative stress in the male reproductive tract. High levels of reactive oxygen species (ROS) may be released by activated leukocytes and/or macrophages, for example, in an inflammatory-infectious process or by the immature sperm cells themselves with excessive cytoplasmic retention [11,42–45].

The close correlation found in this study between major morphological abnormalities and the presence of DNA damage is more likely to be related to the two first hypotheses rather than to an oxidative stressful environment. In fact, it is possible that the two first processes are working synergistically because under the presence of non-orthodox DNA configurations after histone replacement by protamines, apoptotic processes may be triggered. The type and level of DNA damage in each sperm may be responsible in part or in whole for these morphological variations. In cases, such as double sperm heads or reduced heads, meiotic problems of chromosome imbalance or a failure in the second mitotic cytokinesis are likely to be the main producers of abnormal morphology; in both types of sperm abnormalities, a failure of conventional chromatin organization and an increased incidence of apopto-

sis, would be expected. All these considerations, point to the fact that sperm abnormalities could finally produce DNA degradation as part of an apoptotic process to eliminate defective sperm cells. Consequently, most of the morphologically abnormal and DNA-fragmented spermatozoa present in the ejaculate of the bulls studied, would be the result of an "abortive apoptosis"; they may have been signalled for apoptosis but had failed to complete the process.

On the other hand, these morphological abnormalities might potentially have a genetic origin. Sperm DNA damage could be considered as the physiological basis of some morphological defects. Some studies have reported that certain aspects of bull fertility, including morphological abnormalities are probably under genetic control [21,46,47]. It has been proposed that certain major morphologically sperm defects common on DNA damaged spermatozoa are known or suspected to have a genetic basis, at least on occasion [4]. The most frequent, quoted in the literature, are the 'crater defect' [48,49], the 'mini-acrosome sperm defect' [50], the 'stunted tails' including the 'stump defect' [51] and the 'short tail' defect [52].

Since major morphological sperm abnormalities have been associated with reduced fertility [21], their identification in the spermiogram is extremely important for assessing the potential fertility of the ejaculate. This same argument should also apply with respect to DNA-damaged spermatozoa; sperm DNA fragmentation has been reported to have a negative effect on fertility and embryo development [53-56] so its assessment is likely to be useful in fertility evaluation studies. Given the relevance of both parameters it is also important to understand the synergy of the relationship. The relationship presented in this study between major morphological abnormalities and DNA fragmentation suggests that the use of SCD for the simultaneous evaluation of sperm morphology and DNA damage may be useful for the detection of these defective cells. Moreover, as morphologically normal spermatozoa can also present with DNA damage, the inclusion of the assessment of DNA fragmentation in the traditional semen analysis is likely to provide a more thorough evaluation of semen quality and lead to a better ability to predict male fertility in the bull and other animal species. The major advantage of the use of SCD test is that it allows morphology and DNA fragmentation to be conducted simultaneously. This approach is especially powerful when a fine classification of the spermiogram is being used to diagnose testicular function or pathology. In a similar experimental fashion, the SCD test combined with FISH was used to show the incidence of aneuploidies in spermatozoa containing fragmented or intact DNA [31]. A combination of SCDt with other assays is likely to improve semen quality assessment and lead to more informed decisions, when predicting male reproductive potential.

5. Conclusions

In summary, this study showed a clear relationship between morphologically abnormal bull sperm and poor DNA quality. In particular, major sperm abnormalities, that potentially might have a genetic origin or be the result of an abortive apoptotic mechanism, appear to be closely associated with the presence of a highly damaged DNA molecule. As morphologically abnormal spermatozoa are also prone to DNA damage, it is prudent that any assessment of semen quality also incorporate an evaluation of DNA quality. A failure to acknowledge DNA damage in morphologically normal spermatozoa is likely to lead to misdiagnosis of male reproductive potential and an underestimate of male factor infertility. Consequently, we would recommend that assessment of DNA fragmentation be incorporated into routine semen analysis. While the presence of a disrupted sperm DNA molecule is ultimately going to lead to production of a lethal or sub-lethal sperm cell, the aetiology of DNA fragmentation and the full extent of this damage on reproductive potential requires further study.

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Disclosure statement

The authors declare no competing interests.

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