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THE LEVEL OF SPERM DNA FRAGMENTATION IN BULLS OF DIFFERENT BREEDS*

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Abstract

The aim of the present study is to determine the level of spermatozoa with DNA fragmentation in populations of fertile bulls that produce semen for regular insemination, and to identify possible differences in the level of damage between individual breeds. The sperm DNA damages were examined according to Sperm Chromatin Structure Assay method. The semen of 368 ejaculates collected from 187 bulls was used in the study. Bulls of the following breeds were used: Angus Red (AR), Charolais (CH), Polish Black-and-White Holstein (HO), Limousin (LM), Polish Red (RP), Polish Red-and-White Holstein (RW), Simmental (SM) and Polish Red-and-White (SM). The highest mean DFI (3.29%) was found for an Angus Red bull and the lowest (1.49%) for a Polish Red-and-White bull. The highest DFI level found for individual ejaculate was 10.34% (RW bull) and the lowest was 0.26% (HO bull). Statistical differences were found between DFI for the examined breeds.

Key words: bull, sperm DNA fragmentation, sperm chromatin, flow cytometry

The widely used methods for microscopic assessment of sperm quality have a number of important limitations that seriously affect their accuracy and efficiency. Microscopic examination of preparations is a laborious and painstaking process and the final result considerably depends on the experience and degree of tiredness of the technician. The basic parameters determined microscopically include the concentration of semen, the number of motile spermatozoa, and the type of their movement. This assessment is now increasingly based on CASA technology (Computer Assisted Sperm Analysis). Using this technique allows much quicker and more objective results without compromising the quality of semen evaluation. On the other hand, microscopic examination of sperm morphology and biochemistry is even more laborious and the number of evaluated spermatozoa usually does not exceed two hundred.

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In a sense, all of these characteristics are interrelated as they concern evaluation of the possibility of sperm “delivering” genetic material (chromatin) to the oocyte. However, they provide no information on chromatin structure and damage, thus omitting a crucial determinant of male fertility.

During spermatogenesis, sperm chromatin undergoes extensive remodelling. In this process, also known as sperm maturation, chromatin histone proteins are replaced with protamines (small proteins containing large amounts of arginine and cysteine) and sulfhydryl groups (-SH) of these protamines are oxidized to disulfide bridges (-S-S-). These processes are accompanied by a strong condensation of chromatin. The complex processes of chromatin condensation are highly sensitive to disruptive factors. The Sperm Chromatin Structure Assay (SCSA) (Evenson, 1990) is based on the finding that cell DNA shows variable sensitivity to denaturation under low pH conditions. This sensitivity depends on the stage of cell growth and differentiation and on whether these processes are disturbed or subjected to toxic agents. The SCSA method takes advantage of the metachromatic properties of acridine orange (AO).

The SCSA method (but without denaturation) also makes it possible to evaluate the course of changes that chromatin undergoes during spermatogenesis. Yossefi et al. (1994), who used both mBBr and AO to observe the course of sperm chromatin maturation in hamster epididymides, found that oxidation of sulfhydryl groups occurs mainly in the cauda epididymis and is preceded by the main chromatin condensation in the caput epididymis. In addition, the capacity of AO molecules to bind DNA has been found to decrease with advancing oxidation.

Studies of both animal (Ballachey et al., 1987; Bochenek et al., 2001) and human semen (Evenson, 1999; Larson et al., 2000; Zini et al., 2001) showed a close relationship between fertility and the extent of sperm chromatin damage. However, the underlying mechanism of increased chromatin sensitivity to denaturation is still unclear. On the other hand, it has been found that the increased sensitivity of chromatin to denaturation is parallelled by the presence in spermatozoa of loose, short DNA segments resulting from chromatin degradation. Both phenomena are characteristic of apoptosis, an active and programmed form of cell death. This has brought forth a theory (Darzynkiewicz et al., 1997; Januskauskas et al., 2003) that a more complex mechanism may be involved in sperm chromatin damage. Its purpose would be to eliminate spermatozoa with genetic defects. Because this mechanism is activated in the final stage of spermatogenesis, in highly differentiated cells, some apoptotic effectors are inactive by this time. Thus, the whole process is limited to activation of endonucleases that cut DNA into characteristic short fragments. These spermatozoa may show normal mitochondrial activity, normal motility, and in some cases even normal morphology. Whether chromatin damage is significantly related to other sperm characteristics is still a matter of debate. There are reports that either confirm (Zini et al., 2001) or contradict this (Darzynkiewicz et al., 1997; Bochenek et al., 2001).

It is thought, at least in humans, that chromatin damage may be responsible for embryo death in the early stages of embryonic development (Evenson, 1999). These observations provide indirect proof of the hypothesis that there is an apoptotic mechanism behind sperm chromatin damage.

The aim of the present study is to determine the level of spermatozoa with chromatin damage in populations of fertile bulls that produce semen for regular insemination, and to identify possible differences in the level of damage between individual breeds.

Material and methods

The semen of 368 ejaculates collected from 187 bulls was used in the study. Bulls of the following breeds were used: Angus Red (AR), Charolais (CH), Polish Black-and-White Holstein (HO), Limousin (LM), Polish Red (RP), Polish Red-and-White Holstein (RW), Simmental (SM) and Polish Red-and-White (SM). All bulls belonged to the Małopolska Biotechnology Centre in Krasne. Before analysis, the semen frozen in Bioxcell (IMV, France) in standard straws, was thawed at 37ºC for 30 sec. After thawing semen was diluted with PBS to concentration of 1 million spermatozoa/ml. The sperm chromatin damage was examined according to SCSA method (Evenson, 1990). The level of damage was expressed as DNA Fragmentation Index (DFI). This index shows the percentage of spermatozoa with fragmented DNA. Analyses were performed in a DAKO Galaxy flow cytometer. The t test was used for statistical analysis.

Results

The highest mean DFI (3.29%) was found for an Angus Red bull and the lowest (1.49%) for a Polish Red-and-White bull. The highest DFI level found for individual ejaculate was 10.34% (RW bull) and the lowest was 0.26% (HO bull).

The mean DFI values for each of 8 bull breeds are shown in Table 1. Statistical differences between DFI for the examined breeds are shown in Table 2. Statistically significant differences were found between the examined breeds.

Table 1. Sperm DNA fragmentation index (DFI) of bulls of 8 different breeds

Breed of bulls	No. of bulls	No. of ejaculates	Mean DFI	SD
AR	1	3	3.29	0.47
CH	3	14	2.5	1.06
HO	32	67	2.12	1.27
LM	8	24	3.11	1.84
RP	22	27	1.76	0.51
RW	51	97	2.44	1.58
SM	64	128	2.48	1.34
ZR	6	8	1.49	0.6

Breeds: AR – Angus Red; CH – Charolais, HO – Polish Black-and-White HF, LM – Limousin, RP – Polish Red, RW – Polish Red-and-White HF, SM – Simmental, ZR – Polish Red-and-White.

Table 2. Statistical differences of sperm DNA fragmentation index (DFI) between 8 breeds

Bulls' breed	AR	CH	HO	LM	RP	RW	SM	ZR
AR								
CH								
HO								
LM			**					
RP	**	**		**				
RW					*			
SM				*	**			
ZR	**	*		*			*	

* Significance at P<0.05. ** Significance at P<0.01 (t test).
Breeds: AR – Angus Red; CH – Charolais, HO – Polish Black-and-White HF, LM – Limousin, RP – Polish Red, RW – Polish Red-and-White HF, SM – Simmental, ZR – Polish Red-and-White.

Discussion

The results show that the overall level of sperm chromatin damage in the examined bulls is relatively low. This is in contrast to the very well recognized state in humans. It seems that the mechanism of sperm chromatin protamination is responsible for this phenomenon. Bull, stallion, hamster and mouse sperm nuclei contain significantly higher amounts of protamines (about 95%) than human sperm nuclei (about 85%) (Gatewood et al., 1987; Bellve et al., 1988; Bench et al., 1996). In animals, this results in a higher degree of sperm chromatin condensation and thus higher chromatin resistance to fragmentation compared to men (Irvine et al., 2000). Furthermore, bull, boar, ram and cat spermatozoa only contain P1 protamines. Unlike P2 protamines, which are also found in humans, they are more abundant in cysteine residues that contribute to the formation of disulfide bridges, which stabilize sperm chromatin (Corzett et al., 2002). These differences may suggest that animal chromatin is more resistant to damage than in humans. However, despite the extensive number and sophistication of studies concerning the effect of sperm chromatin damage on human fertility, animal research in this area lags far behind.

It is known that sperm chromatin damage/abnormal structure may be also caused by environmental factors such as elevated body temperature (Evenson et al., 2000), toxic agents (Spano et al., 1996; Potts et al., 1999), components of the extender in which semen is stored (Hammadeh et al., 2001), storage conditions (Boe-Hansen et al., 2005) or, in some species, technological procedures to which the semen is subjected (Bochenek et al., 2006). It seems that none of these factors played a role in the study. All examined bulls were kept under the same conditions and all ejaculates were frozen and handled in the same manner.

It is interesting to note detailed observations on the association between sperm chromatin damage and human fertility, which established that the threshold of high risk of infertility in men was very low: DFI = 30% (Larson et al., 2000) or DFI = 25%

(Zini et al., 2001). These surprising results possibly suggest that ejaculates contain a certain number of spermatozoa with chromatin damage that is undetectable by the SCSA method. Unlike in humans, no "infertility threshold" has been established in different species of animals including cattle, to say nothing about the average level of sperm DNA fragmentation in a population of fertile males.

In spite of relatively small differences in DFI between the examined breeds and generally low level of DFI, statistically significant differences between the breeds were found. The question that remains to be answered is whether such differences cause fertility problems in bulls.

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Poziom fragmentacji DNA plemników u buhajów różnych ras

STRESZCZENIE

Celem pracy było ustalenie poziomu uszkodzeń chromatyny w populacjach płodnych buhajów wykorzystywanych do produkcji nasienia na potrzeby regularnej inseminacji oraz stwierdzenia występowania ewentualnych różnic w poziomie uszkodzeń pomiedzy poszczególnymi rasami. Do tego celu wykorzystano metodę Sperm Chromatin Structure Assay. Materiał stanowiło nasienie 368 ejakulatów pochodzących od 187 buhajów 8 ras: angus czerwony (AR), Charolaise (CH), polska HF odmiana czarno-biała (HO), Limousine (LM), polska czerwona (RP), polska HF odmiana czerwono-biała (RW), Simental (SM) oraz polska czerwono-biała (ZR). Stwierdzone dla poszczególnych ejakulatów odsetki plemników z uszkodzoną chromatyną wahały się między DFI = 0,26 (buhaj HO) a DFI = 10,34 (buhaj RW), a średnie dla ras wahały się od 1,49% dla ZR, do 3,29% dla AR. Stwierdzono statystycznie istotne różnice pomiędzy poszczególnymi rasami.