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Three Types of Acrosomal Aberrations of Bull Spermatozoa and their Relation to Fertility

By *Magnus Andersson, Matti Vierula* and *Matti Alanko*

Department of Obstetrics and Gynaecology, College of Veterinary Medicine, Hautjärvi, and Department of Anatomy, University of Kuopio, Finland.

Andersson M., M. Vierula and M. Alanko: Three types of acrosomal aberrations of bull spermatozoa and their relation to fertility. Acta vet. scand. 1990, 31, 175–179. – The effect of acrosomal aberrations of the spermatozoa of Finnish Ayrshire bulls on the corrected non-return rate within 60 days of the first 500 inseminations was studied. The material consisted of sperm samples examined by the artificial insemination societies. All samples had been accepted for use in artificial insemination. One Giemsa-stained slide was studied for each of the 95 bulls concerned. Samples showing distinct acrosomal defects were studied by electron microscopy. Three different types of acrosomal aberration were found. One was obviously associated with subfertility in all 6 bulls in which it was detected.

non-return rate; Ayrshire bull.

Introduction

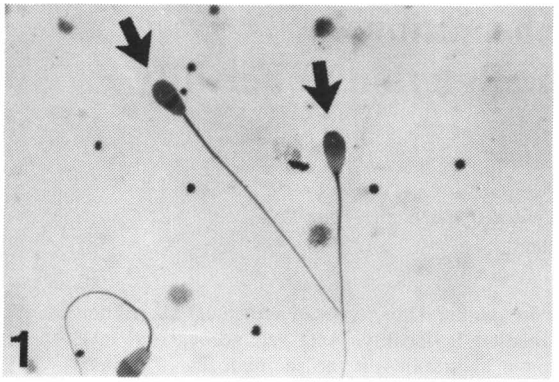
Morphological aberrations of bull sperm acrosomes are associated with diminished fertility, to various degrees, from total sterility (*Teunissen* 1946) to mild subfertility (*Saacke et al.* 1968). In this report, 3 acrosomal aberrations in bull spermatozoa are described. Their possible relation to fertility is evaluated on the basis of the non-return rate.

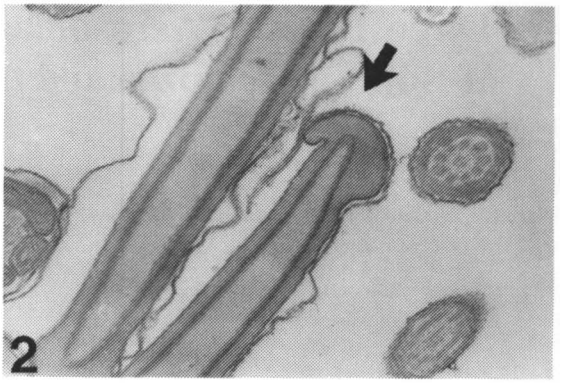
Materials and methods

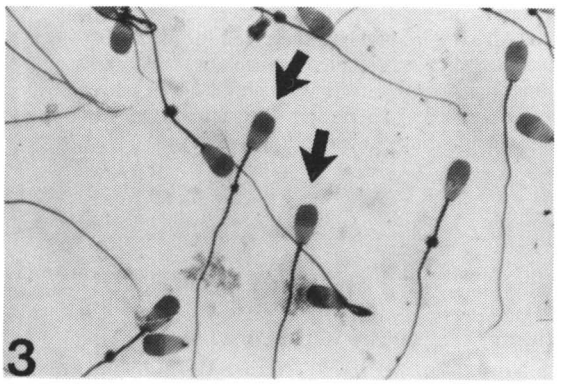
The material consisted of sperm samples which had been accepted for use in artificial insemination (AI) by AI societies. Semen samples from 95 Finnish Ayrshire bulls from 3 AI societies were studied. The corrected non-return rate within 60 d of the first 500 inseminations (NR%) was used as an indicator of fertility. Correction was made according to month of insemination and the average non-return rate for the AI society concerned. Immediately after its collection, the semen was evaluated for motility and density at the AI centres. Smears were prepared from semen diluted with sodium citrate. The smears were stained with Giemsa stain (*Watson* 1975). Acrosomal morphology was evaluated for 100 sperm cells from each sample and a spermiogram was evaluated as described by *Blom* (1981).

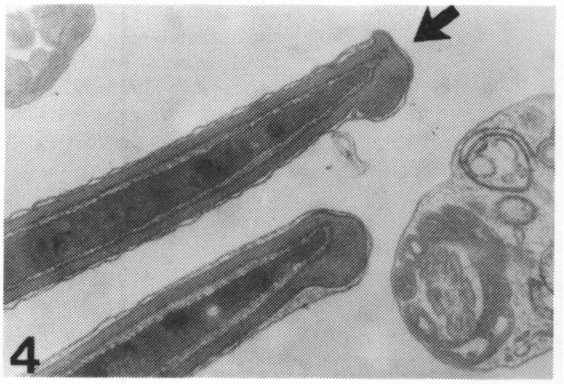
Additional semen samples from bulls exhibiting distinct acrosomal aberrations during light microscopic evaluation were processed for electron microscopy. The spermatozoa were gently washed in phosphate buffered saline, fixed with 2 % glutaraldehyde in 0.15 mol/l sodium cacodylate buffer and postfixed with 1 % osmium tetroxide in the same buffer. The samples were dehydrated in ethanol and propylene oxide and embedded in Epon 812. The ultrathin sections were stained with uranyl acetate and lead citrate.

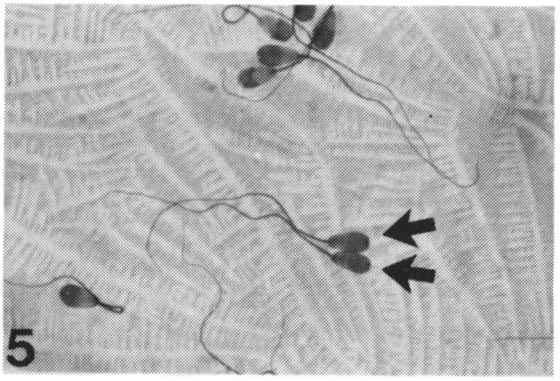
The statistical significance of differences between groups was evaluated using Student's t-test.











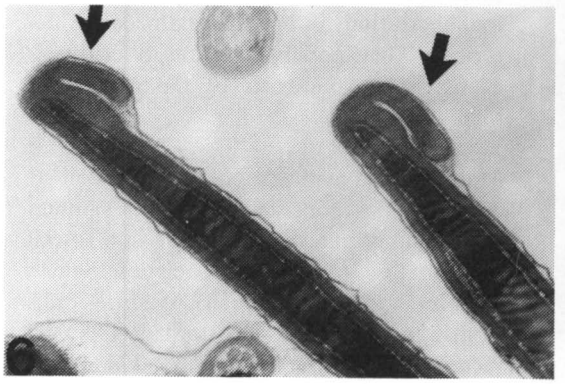


Figure 1. Acrosomes with swollen apical segment. Giemsa × 1000.
Figure 2. Acrosomes with swollen apical segment. × 10.000.
Figure 3. Acrosomes with flattened apical segment. Giemsa × 1000.
Figure 4. Acrosomes with flattened apical segment. × 10.000.
Figure 5. Acrosomes with partial bending of the apical segment. Giemsa × 1000.
Figure 6. Acrosomes with partial bending of the apical segment. × 10.000.

Results

Fifteen bulls with more or less constant acrosomal aberrations were found among the 95 bulls investigated (15.8 %). Two bulls with abnormal acrosomes and 13 bulls with morphologically normal acrosomes had poor general sperm morphology (10 % proximal droplets or more than 15 % of major "primary" sperm defects). These bulls were not included in the fertility evaluation. Three main types of acrosomal aberrations were seen.

Acrosomes with swollen apical segments were found in 6 bulls (category A). This swelling often gave the spermatozoa an asymmetric appearance when they were examined by light microscopy (Fig. 1). Electron micrographs of spermatozoa from this group showed that the apical segment of the acrosome was irregularly swollen. Typically, the acrosomes had a hammerlike appearance in sagittal section (Fig. 2). The frequency of this aberration in the spermiograms of these bulls varied between 22 and 28 %. The mean NR% for 5 bulls with this aberration but an otherwise normal semen picture was 48.8 % (Table 1).

Acrosomes with flattened apical segment were found in 8 bulls (category B). The apical segment of the acrosome appeared to be straightened or notched. In electron micrographs the acrosomes were apically flattened (Figs. 3 and 4). In the spermiograms of these bulls the frequency of the aberration was 8 to 25 %. The mean NR% of 7 bulls with this aberration but an otherwise normal semen picture was 62.3 %.

Acrosomes with partial bending of the apical segment were found in 1 bull (category C). Morphologically, this defect was the most apparent of all the aberrations. With both light and electron microscopy, a local projection backwards was seen in the apical segment of the acrosome. This projection was always bent backwards under the plasma membrane (Figs. 5 and 6). The frequency of the aberration was 30 %. The NR% of this bull 64.6.

Sperm morphology in bulls with acrosomal defects was compared with that in bulls with normal acrosomes. There was no statistically significant difference between sperm defect frequency, including proximal droplets in abnormal and normal acrosome bull categories.

All of the bulls which had acrosomes with swollen apical segments (category A) had an NR% below 60. Only 1 bull with acrosomes with flattened apical segment (category B) had so low an NR%. The 67 bulls with morphologically normal acrosomes (category D) had a mean NR% of 62.3. The NR%

Table 1. Acrosomal morphology and fertility.

Acrosomal morphology	Fertility of bulls: Non-return rate within 60 days mean ± sd	range	No. of xp bulls
A. Acrosomes with swollen apical segment	48.8 ± 5.4	(44–56 %)	5
B. Acrosomes with flattened apical segment	62.3 ± 3.1	(56–66 %)	7
C. Acrosomes with partial bending of the apical segment	64.6	(64.6 %)	1
D. Normal acrosomes	62.3 ± 6.3	(33–70 %)	67

values differed statistically significantly only as between the normal acrosome category and the category A.

Discussion

Mammalian sperm acrosomes obviously have an essential role in penetration of zona pellucida during fertilization (reveiwed by *Talbot* 1985). The acrosome is a membrane-bounded vesicle overlying the anterior part of the sperm nucleus. It contains enzymes necessary for egg penetration. The part of the acrosome projecting beyond the tip of the nucleus is called the apical segment. In this segment, a regionalized structure of acrosomal contents has been observed (*Fawcett* 1975).

In bulls and rams, the apical segment is only a slight swelling on one of the flat sides of the tip of the nucleus. Many acrosomal abnormalities in bulls are associated with sterility (*Teunissen* 1946, *Hancock* 1953, *Blom & Birch-Andersen* 1962) or subfertility (*Saacke et al.* 1968). The acrosomal abnormality associated with sterility is the knobbed-sperm defect, mostly found in Friesian bulls and boars (*Jainudeen & Hafez* 1980). It takes the form of an eccentrically located thickening of the acrosome and affects all or almost all spermatozoa in the ejaculate. This defect is inherited and is probably related to an autosomal recessive gene (*Hancock* 1953). Morphology of acrosomes may be a useful indicator of fertility in domestic animals.

In this study, 3 morphologically different acrosomal aberrations were found in 95 Finnish Ayrshire bulls. One of these defects, a swollen apical segment, was obviously connected with subfertility in all 6 bulls in which it was found. The mean value for NR% in these bulls was 48.8 only. According to AI statistics the mean corrected NR% within 60 d of the first 500 inseminations is about 64 % in Finland. Morphologically, the aberration was fairly unremarkable. The apical segment of the acrosome was irregularly swollen. When examined under the light microscope, the sperm heads seemed more or less asymmetric.

The second acrosomal aberration, a flattened apical segment, appeared not to decrease fertility, on the basis of the NR% values for the bulls concerned.

The third acrosome aberration, partial bending of the apical segment, was found in only 1 bull with a normal NR% (64.6). This aberration was morphologically the most pronounced. Although it appeared to have no effect on fertility, its frequency among spermatozoa was fairly high.

Not all morpholocical aberrations interfere markedly with fertility. In our study 1 acrosome defect, a swollen apical segment, was obviously connected with subfertility.

Theoretically, acrosomal defects could interfere with different stages of the fertilization process. The amounts and actions of enzymes involved in the acrosomal reaction could, in particular, be affected. Some details concerning the function of the acrosome are still unclear. So far, knowledge of the location of sperm hydrolases in the acrosome, and of the actions of individual enzymes in relation to egg penetration is limited. Particular mechanisms possibly involved in different kinds of acrosomal defects need to be studied further.

In our study the frequency of spermatozoa with an abnormal acrosome was less than 35 % in all bulls. The higher percentage of aberrations in the second and third acrosomal aberration categories might have been associated with subfertility.

We would suggest that acrosome morphology is worth evaluation during routine morphological analysis of bovine semen. To improve fertility statistics, the use in AI of

bull semen with acrosomes having swollen apical segments is not recommended.

References

Blom E: Ocena morfologiczna wad plemników buhaja. II. Propozycja nowej klasyfikacji wad plemników. (Morphological evaluation of sperm abnormalities in bulls, 2. A proposal for a new classification of sperm abnormalities). Medycyna Weterynaryjna 1981, *37*, 239–242.

Blom E, Birch-Andersen A: Ultrastructure of the sterilizing "Knobbed-sperm" defect in the bull. Nature, Lond. 1962, *194*, 898–990.

Fawcett DW: The mammalian spermatozoan. Develop. Biol. 1975, *44*, 394–436.

Hancock JL: The spermatozoa of sterile bulls. J. exp. Biol. 1953, *30*, 50–56.

Jainudeen MR, Hafez ESE: Reproductive failure in males. In: Reproduction in Farm Animals. 4th ed. E. S. E. Hafez, Lea & Febiger, Philadelphia 1980, pp 471–493.

Saacke RG, Amann RR, Marshall CE: Acrosomal cap abnormalities of sperm from subfertile bulls. J. Anim. Sci. 1968, *27*, 1391–1400.

Talbot P: Sperm penetration through oocyte investments in mammals. Amer. J. Anat. 1985, *174*, 331–346.

Teunissen GHR: An abnormality of the acrosome in the spermatozoa of a bull. Tidschr. Diergeneesk. 1946, *71*, 292–301.

Watson PF: Use of Giemsa-stain to detect changes in acrosomes of frozen ram spermatozoa. Vet. Rec. 1975, *97*, 12–15.

Sammanfattning

Tre typer av akrosom-avvikelser hos tjurspermier och deras samband med fertiliteten.

I denna undersökning studerades sambandet mellan akrosomavvikelser hos tjurar och det korrigerade resultatet för ejomlöpningsprocenten inom 60 dagar för de första 500 inseminingarna. Tre akrosom-avvikelser konstaterades hos Ay-rasen. Materialet utgjordes av spermaprover som studerats av seminföreningarna och samtliga prover hade godkänts för artificiell inseminering. Ett Giemsa färgat preparat studerades från var och en av de 95 tjurarna. De spermaprov som uppvisade tydliga akrosom-avvikelser undersöktes i elektronmikroskop.

En av dessa akrosom-avvikelser hade ett uppenbart samband med subfertilitet hos alla de 6 tjurar hos vilka avvikelsen konstaterades.

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Reprints may be requested from: M. Andersson, Department of Obstetrics and Gynaecology, College of Veterinary Medicine, 04840 Hautjärvi, Finland.