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A Sperm Midpiece Defect in a Hereford Bull with Variable Semen Quality and Freezability

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Andersen Berg, K., O. Filseth and E. Engeland: A sperm midpiece defect in a Hereford bull with variable semen quality and freezability. Acta vet. scand. 1996, 37, 367-373. – A midpiece sperm defect with a frequency of 25-35% in ejaculates obtained from a Hereford bull with a 60 d non-return rate of 76.4% after careful pre- and post-freeze semen selection was studied in light microscope and by transmission electron microscopy. The defect consisted in a folding and coiling of the distal midpiece characterized by disorganization and irregularity of mitochondria surrounding the axial fiber bundle, combined with retraction of doublet fibers and dislocation and fracturing of these elements and the corresponding dense fibers. Based on examination of the spermatogenic epithelium it was concluded that the alterations in the axial fiber bundle were secondary to those in the mitochondrial sheath. The abnormality appeared to be related to the "Dag-like" defect earlier observed in different breeds.

bull sperm; ultrastructure; classification; fertility.

Introduction

Sperm tail abnormalities are found with varying frequency in virtually all bovine ejaculates. The functional significance in relation to fertility is clearly related to the incidence of defects in the individual bull.

A characteristic deviation from normal development of the midpiece, the "Dag" or "Dag-like" defect has been observed rather commenly in bovine semen with a frequency below 5% *(Barth & Oko* 1989). In a few cases where the incidence exceeded 50%, the defect has been reported to be the cause of subfertility *(Blom* 1966, *Wenkoff* 1978).

In routine assessment of semen quality by use of light microscopy the "Dag" defect appears as a coiling or breakage of the sperm tail, predominantly with the axis of the coiling in the distal part of the midpiece. The defect is usually associated with the cytoplasmic droplet in distal position *(Koefoed-Johnsen & Pedersen* 1971).

The etiology of the "Dag" defect in Danish Jersey breed is reported to involve a recessive gene *(Koefoed-Johnsen et al.* 1980). In some cases the development of the defect is supposed to be related to a malformation of the mitochondrial sheath *(Barth & Oko* 1989). The occurrence of an irregular mitochondrial sheath might seem, however, to be subject to a breed difference, since this feature has not been found in Jersey bulls, but only in cases examined later from Hereford and Swedish Red and White breeds *(Wenkoff* 1978, *Hellmèn* 1980).

The discovery among Hereford bulls of additional cases representing the combination of disorganized mitochondria and an irregular axial fiber bundle would support the idea of a breed difference in regard to pathogenesis as

well as manifestation of this type of midpiece defect.

The present investigation was performed to study the nature of another case of a "Dag-like" defect in a Hereford bull for the purpose of classification. A further objective was to see if the defect had any impact on the fertility.

Materials and methods

The semen was collected from a 1½ year old polled Hereford bull used in artificial insemination. Routine examination of the ejaculates in connection with cryopreservation had revealed a proportion of 25% to 35% of spermatozoa with a characteristic midpiece defect and a varying degree of oligospermia over a periode of three months. The motility was also variable, the fluctuations being inversely related to the incidence of the midpiece defect. Of totally 6195 semen doses 3904 (63.1%) was discharged, 946 doses (15.3%) before and 2958 doses (47.8%) after freezing.

Light microscopy

Smears of semen were made shortly after collection and stained with eosin-nigrosin *(Blom* 1950) for examination in bright field microscope at a magnification of 1000 ×.

Transmission electron microscopy

Semen samples of approx. 0.2 ml were suspended in 3% glutaraldehyde in 0.1 cacodylate buffer (pH 7.4) for prefixation in 36 h at room temperature. The sediment thus formed was washed in the cacodylate buffer for 2×10 min and fixed in 2% OsO_4 in 0.1 M cacodylate buffer for 2 h at 20°C. Subsequently it was washed once more in the cacodylate buffer for another 2×10 min.

Further, the material was dehydrated in ascending series of ethanol and propylene oxide and then infiltrated with LX 112-propylene oxide (1:1 for 1 h and 1:3 overnight) before it was embedded in LX 112. Ultrathin sections were made with an RMC ultrotome.

At slaughtering of the bull small pieces of tissue from testis and epididymis were allocated for electron microscopy immediately post mortem. Preparation, embedding and sectioning were performed by the procedure described above for semen.

Ultrastrucural studies of sperm cells and spermatogenic epithelium were done by use of a Jeol JEM 100 S Elmiscope.

Results

The appearance of the defect examined in bright field microscope was characterized by an irregular mitochondrial sheath combined with a folded, coiled or twisted distal midpiece (Fig. 1). The reflexion of the tail was often associated with a distal cytoplasmic droplet. The proportion of affected spermatozoa was estimated to 31% in the smears examined from the ejaculate. In epididymal spermatoza the frequency of folded and reflected tails was definitely lower, especially in those from the caput epididymidis (<10%).

The ultrastructural manifestations of the defect invariously consisted in abscence of mitochondria in greater or smaller parts of the midpiece (Fig. 2a). In proximal sections from this portion of the tail the axial fiber bundle generally appeared to be normal (Fig. 2b), even if abscence of doublets could be encountered (Fig. 2c). In sections of the coiled distal part of the midpiece both the axonemal doublets and the corresponding dense fibers were often missing, or found dislocated in the residual cytoplasm (Fig. 3). In some cases axial fibers were fractured and protruded through the defects in the mitochondrial sheath (Fig. 4). In the axonemal complex both the smaller group of fibers, doublet 9, 1 and 2 (Fig. 2c) and the larger group, doublets 4,

5, 6, and 7 (Fig. 5) could be missing in transverse sections of the tail. Occasionally only doublet 3 and 8 were left in addition to the 2 central microtubules.

In sections of the spermatogenic epithelium late spermatids just before spermiation displayed an aberrant development of the mitochondrial sheath characterized by a retardation in the arrangement and condensation of mitochondria (Figs. 6, 7). The developing midpiece was enclosed in considerable amounts of residual cytoplasm which also contained deposits of granular material (Fig. 7). No deviation from the normal differentiation of the axial fiber bundle was observed.

Semen collected within the period of investigation was tested for fertility in a routine AI program. Totally 343 first inseminations were recorded, and the 60 d. non return rate was 76.4%.

Discussion

The structural characteristics of the described sperm abnormality including both the misalignment of mitochondria and the malformation of the axial fiber bundle clearly show that it is to be classified as the type of "Daglike" defect found earlier in the Hereford and Swedish Red and White breeds *(Wenkow* 1978, *Hellmèn* 1980). It would thus seem to be essensially different from the "Dag" defect described in Jersey bulls where the pathogenesis was tentatively related to premature release of enzymes from lysosomes in the cytoplasmic droplet, possibly taking place as a consequence of disturbances in the epididymal environment *(Blom & Birch-Andersen* 1966). In Jersey bulls weakness of the dense fiber due to zinc excess has also been suggested as a possible cause *(Blom & Wolstrup* 1976).

In the present study the observed malalignment of the mitochondria in the developing midpiece of spermatids just prior to spermiation may account for the disorganization of the axial bundle and the coiling of the tail registered later in epididymal and particularly in ejaculated spermatozoa. A normally developed mitochondrial sheath is supposed to provide structural support to the axial fibers in the midpiece region. Without this support the flagellar motility gained during epididymal transit may therefore bring about the coiling and reflexion of the tail at the distal midpiece level. A malformation of the normal framework constituted by the mitochondrial sheath might in this way result in a retraction of the doublets observed as a lack of these elements in cross sections of the distal midpiece and the principal piece, and in a dislocation and eventual fractioning of the doublets as well as the corresponding dense fibers. The fact that both the group of fibers 4, 5, 6 and 7 and that of 9, 1 and 2 are involved, indicates that the midpiece can be reflected to either side of the plane through the central pair of tubules and axial fibers 3 and 8.

As a deviation from the normal condensation and arrangement of mitochondria appears to be involved in the pathogenesis of the defect, factors influencing the differentiation of the mitochondrial sheath is of etiological interest. Normally the mitochondria migrate into the region surrounding the dense fibers of the axial bundle as the annulus moves posteriorly in late maturation phase of spermateleosis *(Burgos et al.* 1970), and in the bull the number of gyres in the sheath is about 10 *(Fawcett* 1970). Concurrently with this assembly in the midpiece, the mitochondria normally undergo an internal reorganization *(Andrè* 1962). The genetic information which controls the differentiation is carried by preformed RNA, as the synthesis of this factor declines in spermatogenic cells after pachytene and ceases totally in connection with the flattening of the spermatid nucleus *(Loir* 1971, *Fulcher et al.* 1993, *Saunders et al.* 1993). Concomitantly with spermatid differen-

tiation the RNA is accumulated in the cytoplasm and finally eliminated in the residual body *(Monesi* 1971). The granular material seen in the vicinity of the developing midpiece just before spermiation might consist of or be derrived from RNA, since it is generally associated with the chromatoid body, which is belived to be of nucleolar origin *(Commings & Okada* 1972).

In addition to genetic factors, also other conditions such as testicular concentration of selenium may play a role in the development of of the mitochondrial sheath *(Pallini & Bacci* 1979). Selenium deficiency is supposed to induce changes in sperm mitochondria due to lack of Se covalent bonding or cross-linking *(Wallace et al.* 1983). In rodents inadequate supply of selenium results in malformation of the mitochondrial sheath, similar to that encountered following gossypol treatment *(Oko & Hrudka* 1982). Glossypol is further reported to induce the same type of granular material deposits in combination with disarrangement of the mitochondrial sheath as observed in the present investigation *(Barth & Oko* 1989).

The effect of the described abnormality on fertility is difficult to evaluate. No exact registration of the incidence of the defect had been performed for semen used in artificial insemination. The NR rate recorded was, however, clearly above the average for the bulls in the AI centre,and even if more than half of the semen collected during the actual period was discharged for different reasons, the frequency must still have been at least 25%.

Apparently, the tolerance level of this defect is rather high. This is probably also to be expected, since the affected spermatozoa are not likely to penetrate the zona pellucida and initiate a zona reaction excluding other sperm cells.

Figure 1. Photomicrograph of ejaculated bovine spermatozoa with a flagellar defect. The two affected sperm cells (A and B) have an irregular mitochondrial sheath and one of them a reflexion of the tail at the level of the distal midpiece (arrow). × 1,000.

Figures 2–7. Transmission electron micrographs of ejaculated spermatozoa and late spermatids from a bull with a sperm tail defect.
Pl = plasmalemma, M = mitochondria, Fs = fibrous sheath, Df = dense fibers, Md = microtubule doublets, Cp = central pair of single microtubules, S = Sertoli cell cytoplasm.

Figure 2 a,b,c. Longitudinal (a) and transverse (b and c) sections of the midpiece. In fig a and b the defect in the mitochondrial sheath (arrow) is not combined with any detectable disarrangement of the axial fiber bundle wheras in fig. c doublet 9, 1 and 2 are missing. × 45,000.

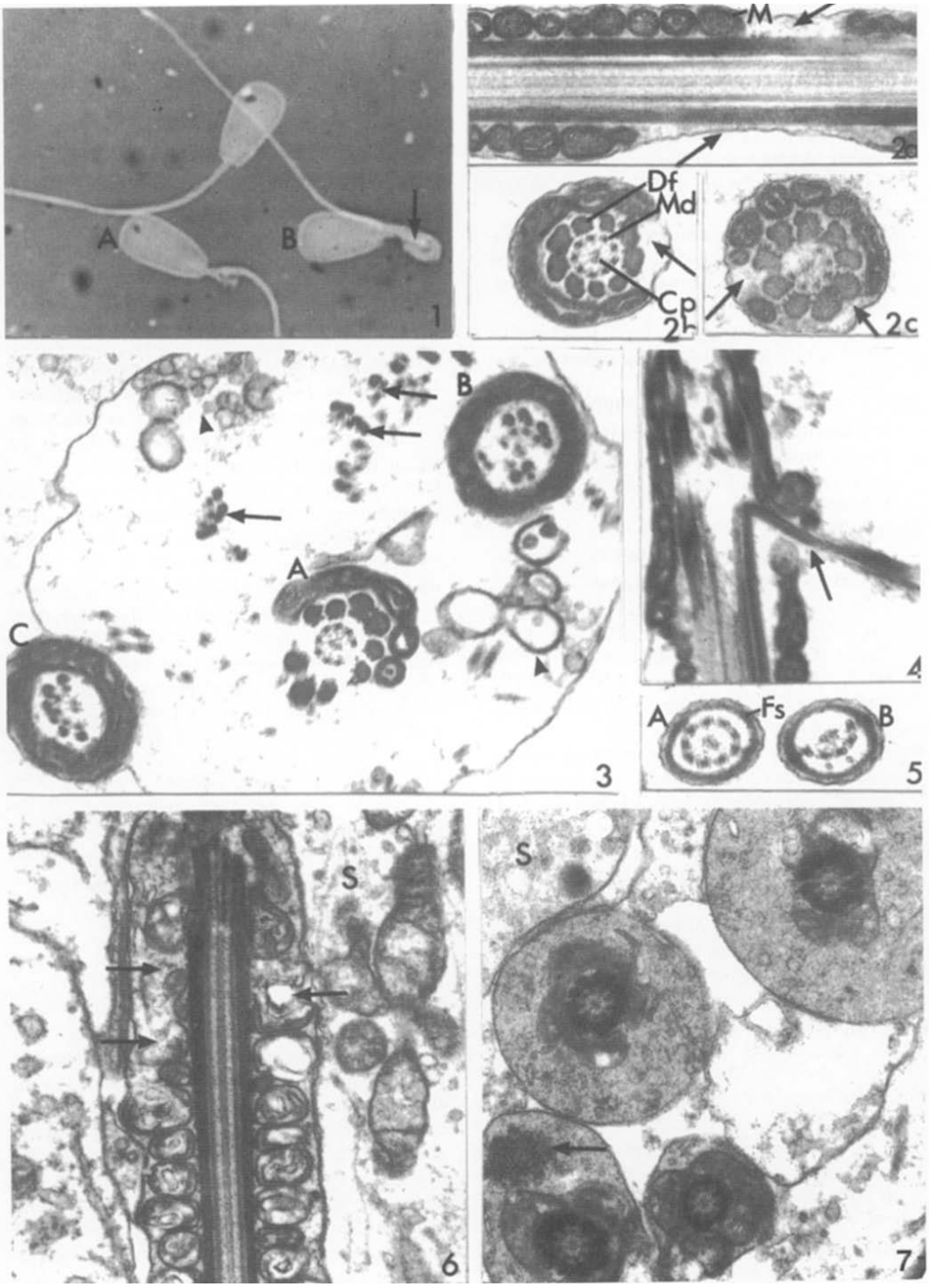
Figure 3. Three transverse sections of a coiled midpiece enclosed in a cytoplasmic droplet and encapsulated in a common plasmalemma (Pl). Cross section A exhibits a defect mitochondrial sheath, asymmetrically arranged dense fibers and a missing doublet 8 while in section B and C several doublets and dense fibers are missing. Dislocated axial fibers (arrows) can be seen scattered arround in the residual cytoplasm, which also contains vesicles and different membranous structures (arrowheads). × 45,000.

Figure 4. Longitudinal section of a proximal midpiece with splitting and fracture of a doublet and the corresponding dense fiber, both protruding through a defect in the mitochondrial sheath (arrow). × 45,000.

Figure 5. Transverse sections of the distal part of two principal pieces, one (A) with a normal axonemal complex, the other (B) lacking doublets 4, 5, 6 and 7 and with 2 single microtubules interposed between the axonemal complex and the surrounding fibrous sheath. × 45,000.

Figure 6. Longitudinal section of a late spermatid displaying abnormal development of the mitochondrial sheath (arrows). × 20,000.

Figure 7. Transverse sections of developing midpieces of late spermatids. The organization of mitochondria, which are swollen and dilatated, is clearly irregular. Accumulations of granular material (arrow) can be seen in the residual cytoplasm. × 12,000.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References

Andrè J: Contribution à la connaissance du chondriome. Etude de ses modifications ultrastructurales pendant la spermatogenèse. J. Ultrastruct. Res. 1962, Suppl. 3, 1-185.

Barth AD, Oko RJ: Defects of the sperm tail. In: Abnormal Morphology of Bovine Sperm. IS Iowa State University Press/Ames, Iowa, 1989 pp 214-270.

Blom E: A simple rapid staining method for differentiation between live and dead sperm cells by means of eosin and nigrosin. Nord. Vet. Med. 1950, *2,* 58-61.

Blom E: A new sterilizing and heridity defect (the "Dag" defect) located in the bull sperm tail. Nature 1966, *209,* 739-740.

Blom E, Birch-Andersen A: The ultrastructure of a new heriditary sterilizing defect (the "Dag" defect) in the bull sperm tail. Proc. 5. Congr. Fertil. Steril. Stockholm. Excerpta Medica) 1966, *133,* 602-605.

Blom E, Wolstrup C: Zink as a possible causal factor in the sterilizing sperm tail defect, the "Dag" defect, in Jersey bulls. Nord. Vet. Med. 1976, *28,* 515-518.

Burgos MH, Vitale-Calpe R, Aoki A: Fine structure of the testis and its functional significance. In: Johnson AD, Gomes WR, Vandemark NL (eds): The Testis. Academic Press, New York and London, 1970, vol. I, pp 551-649.

Commings DE, Okada TA: The chromatoid body in mouse spermatogenesis: Evidence that it may be formed by extrusion of nucleolar components. J. Ultrastruct. Res. 1972, *39,* 15-23.

Fawcett DW: A comparative view of sperm ultrastructure. Biol. Reprod. 1970, Suppl. 2, 90-127.

Fulcher KD, Welch JE, Davis CM, O'Brian DA, Eddy EM: Characterization of laminin receptor messenger ribonucleic acid and protein expression in mouse spermatogenic cells. Biol. Reprod. 1993, *48,* 674-682.

Hellmèn E, Plöen L, Settergren I, Nicander L: Middle piece defects of testicular origin in bull sperm. Nord. Vet. Med. 1980, *32,* 423-426.

Koefoed-Johnsen HH, Andersen JB, Andresen E, Blom E, Philipsen H: The Dag defect of the tail of the bull sperm. Studies on the inheritance and pathogenesis. Theriogenology 1980, *14,* 471-475.

Loir M: Protein and ribonucleic acid metabolism in spermatocytes and spermatids of the ram (Ovis aries). I. Incorporation and fate of ^{3}H-uridine. II. Incorporation and fate of ^{3}H-lysine, ^{3}H-arginine and ^{35}S-cystine. Ann. Biol. Anim. Biochem. Biophys. 1971, *12,* 203-219, 411-429.

Monesi V: Synthetic activities during spermatogenesis in the mouse. Exptl. Cell Res. 1965, *39,* 197-224.

Oko R, Hrudka F: Segemental aplacia of the mitochondrial sheath and sequelae induced by gossypol in rat spermatozoa. Biol. Reprod. 1982, *26,* 183-195.

Pallini V, Bacci E: Bull sperm selenium is bound to a structural protein of mitochondria. J. Submicrosc. Cytol. 1979, *11,* 165-170.

Saunders PTK, Millar MR, West AP, Sharpe RM: Mitochondrial cytochrome c oxidase II messenger ribonucleic acid is expressed in pachytene spermatocytes at high levels and in a stage-dependent manner during spermatogenesis in the rat. Biol. Reprod. 1993, *48,* 57-67.

Wallac E, Cooper GW, Calvin: Effects of selenium deficiency on the shape and arrangement of rodent mitochondria. Gamete Res. 1983, *4,* 389-399.

Wenkoff MS: A sperm mid-piece defect of epididymal origin in two Hereford bulls. Theriogenology 1978, *10,* 4, 275-281.

Wolley DM: The midpiece of the mouse spermatozoon: Its form and development as seen by surface replication. J. Cell Sci. 1970, *6,* 865-879.

Sammendrag

Mellomstykke-defekt hos spermier fra en Hereford-okse med varierende sædkvalitet og spermiefrysbarhet.

En spermie-defekt lokalisert til flagellens mellomstykke ble påvist med en frekvens på 25-35% i ejakulatene fra en Hereford-okse som ble brukt i produksjon av frossen sæd og hadde en 60 d NR% på 76,4 etter sterk seleksjon av sæden både før og etter frysing. Defekten, som ble undersøkt både i lys- og elektronmikroskop, besto i en uregelmessig organisering av mellomstykkets mitokondrier og i en retraksjon av de dobbelte mikrotubuli med påfølgende dislokasjon og fraktur både av disse og av de ytre fibre og bøyning av spermiehalen. Ultrastrukturelle undersøkelser av det spermatogenetiske epitel tydet på at forandringene i flagellens mikrotubuli og ytre fibre var sekundære til dem som ble påvist i mito-

kondriekappen, og at bøyningen av halen oppsto ved induksjon av motilitet under transport gjennom bitestikkelen. Klassifiseringen av defekten i forhold til de tidligere beskrevne "Dag" og "Dag-liknende" defekter hos ulike storferaser og defektens betydning for fruktbarheten blir diskutert.

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