Acta vet. scand. 1985, *26*, 513—520.

From the Department of Anatomy and Histology, and the Department of Obstetrics and Gynaecology, Faculty of Veterinary Medicine, Swedish University of Agricultural Sciences, Uppsala.

ABAXIAL IMPLANTATION OF THE MIDDLE PIECE IN SPERMATOZOA AND SPERMATIDS IN RELATED STERILE BOARS

By

G. Thilander, I. Settergren and *L. Plöen*

THILANDER, G., I. SETTERGREN and L. PLÖEN: *Abaxial implantation of the middle piece in spermatozoa and spermatids in related sterile boars.* Acta vet. scand. 1985, *26*, 513—520. — Spermatozoa and developing spermatids showing neck region abnormalities have been studied in material from 2 genetically related boars. In both boars the defects were abaxial implantation of the tails and lack of substance in the neck region. In many spermatozoa, a too wide space between the capitulum and the basal plate was more pronounced in epididymal spermatozoa compaired to testicular material. This implies that the defect aggravated, and might be connected with the migration of the cytoplasmic droplet in the epididymis. Since the defects were observed in spermatids, it its concluded that the defects were heriditary.

This conclusion was further supported by the observation of similar defects in 6 other related boars, examined by light microscopy only.

neckpiece defect; testis.

Several defects in the neck region of sperm have been described in different species. One of these defects, abaxial implantation of the tail, was found in very low numbers in normal bulls by *Lagerlöf* (1934), *Blom* (1950) and *Rao* (1971). If the defect occurs in a high number of the sperm there may be no motility (*Thilander et al.* 1985). Even if the defect is less frequent the fertility of the bull may be lowered (*Onstad* 1963, *Roslanowski* 1969). In the stallion abaxial implantation of the sperm tail seems to be a normal condition and does not affect fertility.

In boars *Hancock* (1957) reported that abaxial implantation of the sperm tail was common in normal animals. *Müller &*

Brandl (1975) found in boars used for A.I. service a frequency of 25—75 % abaxially implanted tails without any effect on fertility. On the other hand *Paufler* (1965) regarded abaxial implantation of the tail as a cause of infertility. If abaxial implantation of the sperm tail is defined as a deviation of at least the breadth of the midpiece from the midsagittal plane of the sperm head this defect is uncommon in normal boars (*Bane*, personal communication).

In the present report neck piece abnormalities — in the light microscope detected due to abaxial implantation of the sperm tail — from 2 related boars are described. The similarities in the fine structure of the abnormal neck pieces imply that the defects are hereditary.

MATERIAL AND METHODS

Animals

Two boars of Swedish Landrace were sold for breeding but turned out to be infertile. The first of them 354 H was used in 2 herds and served in all 13 gilts repeatedly without getting any gilt pregnant. The other boar, 393 H, served 6 gilts during 2 months but none became pregnant. Both boars were brought to the Dept. of Obstetrics and Gynaecology, College of Veterinary Medicine, Uppsala, where they were examined for some months before being slaughtered.

The boars came from the same herd. Sow 916 was the dam of 354 H and the dam's dam of 393 H. Several other boars related to sow 916 have had the same sperm defect as the boars described here.

Methods

After slaughter material was taken from the testes and epididymides of both boars. For light microscopy material was fixed in Bouin's fluid and embedded in paraffine wax. For electron microscopy, small pieces of tissue were taken from the testes and from 3 different regions of the epididymides (caput, corpus and cauda). The material was fixed in 3 % glutaraldehyde in 0.067 mol/l cacodylate buffer, pH = 7.2. Also sperm from the rete testis (boar 354 H) and from corpus and cauda epididymidis (boar 393 H) were fixed in the same fixative. The material was then treated with OsO_4 for 1 h, dehydrated and embedded in Epon. The suspension of sperm was gently centrifuged and re-

suspended several times during fixation, dehydration and embedding. Semithin sections were stained with buffered toluidine blue and suitable areas were selected. Thin sections were cut with diamond knives on an LKB Ultrotome, stained with uranyl acetate and lead citrate and examined in a Philips EM 201 electron microscope.

RESULTS

Clinical and light microscopical examination of the semen

Semen was collected 3 times from each boar and the results of the examinations are shown in Table 1. As can be seen from the table the main abnormality was abaxial implantation of the tail which was found in almost all sperm. The motility was also low.

Gross examination of the sexual organs before and after slaughter did not reveal any abnormalities.

Table 1. Mean and range of some semen parameters in 3 ejaculates from each boar.

	Boar	
	354 H	393 H
Volume, ml	125 (100—150)	135 (84—220)
Motility %	50 (45— 55)	45 (40— 50)
Concentration $\times$ 1000/mm^3	285 (260—410)	457 (350—620)
Abnormal spermheads %	3.3 (3.2— 3.6)	3.4 (3— 4)
Abaxial implantation of tail %	96.7 (95— 98)	96.8 (96.4— 97)
Proximal cytoplasmic droplet %	1.7 (1— 2)	5.3 (3— 7)

Ultrastructural examination of testes and epididymal sperm

In epididymal spermatozoa the dominating defect was paucity of material in the neck piece. In its mildest form only fragments of the striated columns were missing and the capitulum was poorly developed (Figs. 1 and 2). This occurred on only one side of the neck piece. In such spermatozoa the capitulum and the basal plate were abnormally separated and the entire neck piece appeared widened.

Other spermatozoa showed more severe defects with absence of some of the striated columns. In some instances there was no contact between the remining columns and the capitulum (Fig. 3). Moreover, the capitulum was irregularly developed and hence

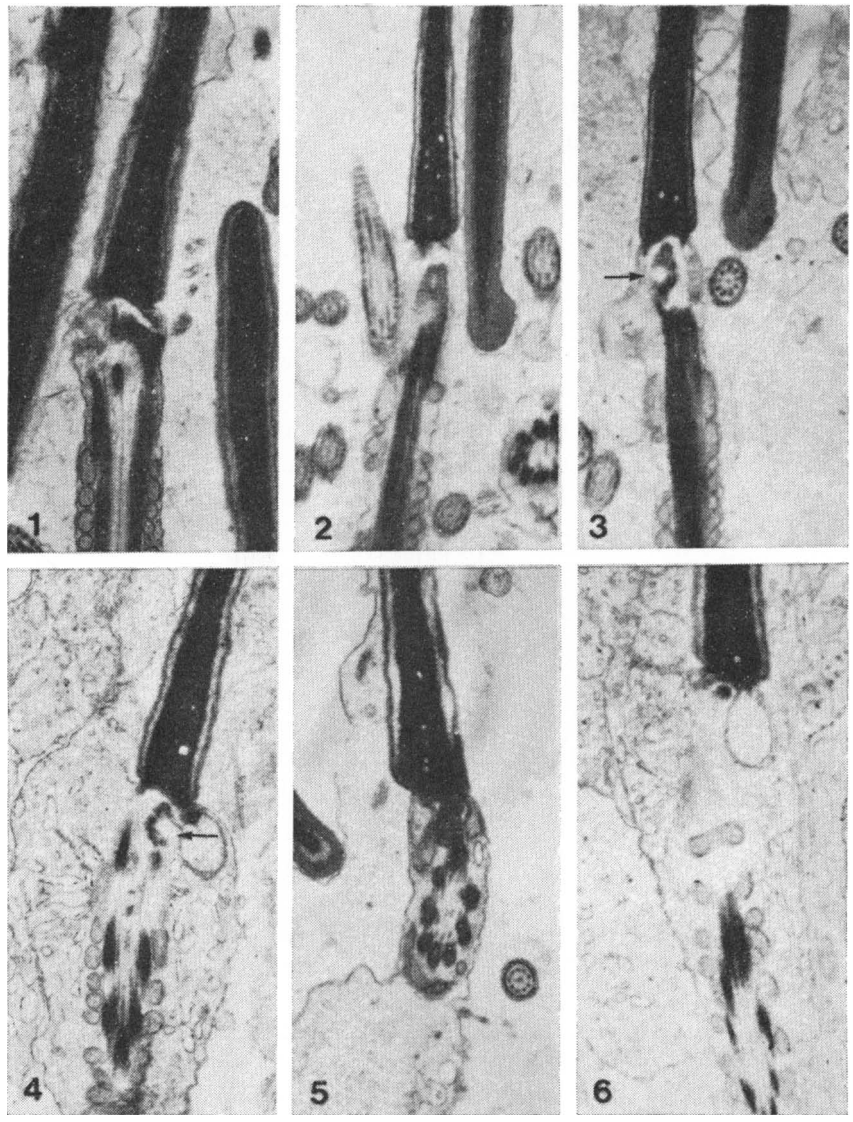

Figure 1. Spermatozoon from corpus epididymidis. Fragments of the striated columns are missing and the capitulum is not completely developed. (× 17,500).

Figure 2. Spermatozoon from corpus epididymidis. This defect is similar to that shown in Fig. 1. Note the abnormally wide space between the basal plate and the capitulum. (× 17,500).

Figure 3. Spermatozoon from corpus epididymidis with almost complete absence of striated columns. Note the missing triplets in the proximal centriole (arrow). (× 17,500).

Figure 4. Late spermatid. The defect is similar to that shown in a spermatozoon in Fig. 3. (× 17,500).

Figure 5. Spermatozoon from corpus epididymidis. The normally developed capitulum has an abaxial implantation to the basal plate and is protruding towards the plasmalemma of the neck piece (× 17,500).

Figure 6. Late spermatid. The basal plate is too narrow and obliquely situated, which gives the capitulum an abaxial implantation. The tail forms an angle with the spermatid head. (× 17,500).

the implantation toward the basal plate was oblique. The space between the basal plate and the capitulum was widened as was the entire neck piece.

The most severely altered spermatozoa were almost devoid of striated columns and hence the dense fibers of the middle piece had no anterior insertion (Fig. 3). The proximal centriole was abnormal, some of the triplets were missing. The capitulum was poorly developed and thus the implantation towards the basal plate was incomplete and the space between these structures was very wide; the tail appeared to be connected to the head only by the intact plasmalemma (Figs. 3 and 4). Also these neck pieces were wider than normally.

The above described abnormalities were also seen in late spermatids.

Another common defect was abaxial implantation of the capitulum towards the basal plate (Fig. 5). In some of these spermatozoa the basal plate appeared normal but the capitulum was poorly developed, and much smaller than the basal plate. Here the small capitulum was abaxially dislocated. In other spermatozoa the posterior part of the nucleus was narrow and the basal plate was not fully developed and obliquely situated. The normally sized capitulum was thus only partially in contact with the basal plate; the surplus part was protruding towards the plasmalemma of the neck piece.

In still other cases both the basal plate and the capitulum appeared normal but the implantation was abnormal and showed an abaxial dislocation of the neck piece. The tail formed an angle with the head. These defects were also observed in late spermatids (Fig. 6).

Apart from the described abnormalities only few other defects involving the acrosome, the nucleus and the tail were observed in the electron microscope. Bent tail occurred, and sometimes some of the doublets and dense outer fibers of the axoneme (generally 9, 1 and 2) were missing. In some spermatozoa the mitochondrial sheaths were discontinuous; one or more mitochondria were absent.

DISCUSSION

The structure of the neck region of mammalian spermatozoa is very complex and shows species differences. It is comprised of a basal plate and a connecting piece (cf. *Nicander & Bane*

1962a, 1962b, *Fawcett & Phillips* 1969, 1970, *Fawcett* 1975). Parts of the caudally directed nuclear envelope is thickened, forming the basal plate, which is connected to the capitulum by a number of microfilaments traversing a space of 40—60 nm. Almost perpendicular to the proximal centriole and parallel to the long axis of the sperm is the distal centriole, which in bulls and boars, disintegrates during formation of the striated columns. The 2 centrioles together form the striated columns, while the capitulum is formed by the proximal centriole alone (cf. *Fawcett & Phillips* 1969). Defects of the sperm neck region are often associated with severely impaired fertility or sterility (cf. *Hancock* 1955, *Settergren & Nicander* 1968, *Blom & Birch-Andersen* 1970, *Thilander et al.* 1985). Disintegration of spermatozoa has been described as a sterilizing defect in bulls (cf. *Hancock* 1955, *Settergren & Nicander* 1968, *Blom & Birch-Andersen* 1970). All these authors found that the separation between head and tail took part in the caput epididymidis while spermatids seemed to be intact. According to *Hancock* (1955), *Settergren & Nicander* (1968), the disintegration of the spermatozoa was associated to the migration of the cytoplasmic droplet occurring in the same region. *Settergren & Nicander* (1968) also found that non-disintegrated spermatozoa showed a marked abaxial implantation, in all of the disintegrated spermatozoa, one of the striated columns was poorly developed or missing. *Blom & Birch-Andersen* (1970) have reported a lack of development in the basal plate that, according to the severeness, gave rise to either an abaxial implantation of the capitulum or a totally disorganized implantation region. *Thilander et al.* (1985) found neck region abnormalities in bull spermatozoa caused by asymmetry of the caudal part the nucleus, which affected the implantation fossa and the development of the connecting piece.

The most common defect in the present material, i.e. the lack of substance in the neck region, was observed already in spermatids. Also the abaxially implantated sperm tails were observed in spermatids. Other defects, e.g. the too wide space between the capitulum and the basal plate was also noticed in testicular material, but was more pronounced in epididymal spermatozoa, implying that the defect had aggravated during epididymal transit. This aggravation might be connected with the migration of the cytoplasmic droplet in the epididymis. The observed lack of contact between the striated columns and the

capitulum in some epididymal spermatozoa was not observed in spermatids, it is thus possible that this defect either became visible or arose during epididymal transit.

It thus seems that all defects observed were of testicular origin, but that some of the defects were aggravated further during epididymal transit. The fact that the 2 boars were genetically related supports the theory that they had an inherited disposition for development of neck piece defects. The same specific light microscopical abnormality has also been observed in 6 other related boars (*Settergren*, unpublished observations), 4 of which have been tested and found sterile.

ACKNOWLEDGEMENT

This study was supported by a grant from the Swedish Council for Forestry and Agricultural Research.

REFERENCES

Blom, E.: Om bedømmelsen af tyresperma (On the evaluation of bull semen). Diss., Copenhagen 1950, 223 pp.

Blom, E. & A. Birch-Andersen: Ultrastructure of the "decapitated sperm defect" in Guernsey bulls. J. Reprod. Fertil. 1970, *23*, 67—72.

Fawcett, D. W.: The mammalian spermatozoon. Dev. Biol. 1975, *44*, 394—436.

Fawcett, D. W. & D. M. Phillips: The fine structure and development of the neck region of the mammalian spermatozoon. Anat. Rec. 1969, *165*, 153—184.

Fawcett, D. W. & D. M. Phillips: Recent observations on the ultrastructure and development of the mammalian spermatozoon. In: B. Baccetti (Ed.), Comparative Spermatology. Academic Press, New York, NY. 1970, p. 13—28.

Hancock, J. L.: The disintegration of bull spermatozoa. Vet. Rec. 1955, *67*, 825—826.

Hancock, J. L.: The morphology of boar spermatozoa. J. R. microsc. Soc. 1957, *76*, 84—97.

Lagerlöf, N.: Morphologische Undersuchungen über Veränderungen im Spermabild und in den hoden bei Bullen mit vermindeter oder aufgehobener Fertilität (Morphological studies of changes in the spermiogram and the testes of bulls with low fertility and infertility). Acta path. microbiol. scand. Suppl. 1934, *19*, 254 pp.

Müller, E. & G. Brandl: Untersuchungen über die Motilität und Morphologie von Eberspermatozoen (Studies on the motility and morphology of boar spermatozoa). Dtsch. Tierärztl. Wschr. 1975, *82*, 153—155.

Nicander, L. & A. Bane: Fine structure of boar spermatozoa. Z. Zellforsch. 1962a, *57*, 390—405.

Nicander, L. & A. Bane: New observations on the fine structure of spermatozoa. Int. J. Fertil. 1962b, 7, 339—344.

Onstad, O.: Abaksial tillheftning av spermiens mellemstycke (Abaxial implantation of the sperm middle piece). Nord. Vet.-Med. 1963, *15*, 92—96.

Paufler, S.: Fortplanzungsstörungen bei Schweinen (Reproductive disturbances in swine). In: Die Schweinebezamung, Ed. D. Smidt, Verlag Shaper, Hannover 1965, p. 32—116.

Rao, A. R.: Changes in the morphology of sperm during their passage through the genital tract in bulls with normal and impaired spermatogenesis. Thesis, Stockholm 1971, 88 pp.

Roslanowski, K.: Morphological abnormality in the structure of spermatozoa of two generations of bulls. Genet. Pol. 1969, *10*, 152—155.

Settergren, I. & L. Nicander: Ultrastructure of disintegrated bull sperm. 6th Congr. Int. Reprod. Anim. Insem. Artif. Paris, Vol. 1, 1968, p. 191—194.

Thilander, G., I. Settergren & L. Plöen: Abnormalities of testicular origin in the neck region of bull spermatozoa. Anim. Reprod. Sci. 1985, *8*, 151—157.

SAMMANFATTNING

Abaxial mittstycksimplantation hos spermier och spermatider hos besläktade sterila galtar.

Spermier med abaxialt infästade mittstycken från 2 galtar som var helbröder har studerats. Elektronmikroskopiska undersökningar visade att material saknades i spermiernas halsstycken, samt bekräftade den abaxiala spermiesvansinfästningen. En annan vanligt förekommande defekt hos spermierna var för brett mellanrum mellan capitulum och "striated columns". Denna defekt var mer markant hos bitestikelspermier än hos spermatider, vilket talar för att defekten aggraverar under passagen genom bitestikeln och kan vara kopplad till cytoplasmadroppens vandring längs spermiens mittstycke. Det faktum att sådana defekter observerades redan hos avlånga spermatider, talar för att de är ärftliga. Denna slutsats styrks av iakttagelsen av liknande defekter hos 6 besläktade galtar.

(Received August 26, 1985).

Reprints may be requested from: G. Thilander, Box 7011, S-75007 Uppsala, Sweden.