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STICKY CHROMOSOMES AS A CAUSE OF TESTICULAR HYPOPLASIA IN BULLS

By

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By testicular hypoplasia is generally meant defective or incomplete development of the seminiferous epithelium. In Swedish Highland cattle, for example, there is a type of testicular hypoplasia reaching such a degree that affected testicles are incapable of differentiating spermiogenic cells. This particular type of hypoplasia has been well characterised clinically (*Lagerlöf et al.* 1934, 1951, 1956a, 1956b) and genetically (*Eriksson* 1939, 1949). Furthermore, the morphological defects in the seminiferous epithelium are clearly defined (see Lagerlöf). Types af testicular hypoplasia with differentiated seminiferous epithelium, on the other hand, have offered much greater difficulties in the interpretation of the finer details in their histological appearance (*Williams* 1921, *Lagerlöf* 1934, 1948, *Fincher et al.* 1942, *Haq* 1949, *Gibbons* 1953, and *van der Sluis* 1953, among others). If the spermiogenic cells have differentiated but the epithelium is incapable of forming sperm, then the fault must lie in the cell divisions of spermiogenesis or in the transformation of spermids to sperms. The only approach to the study of the cell divisions (A-spermatogonia, B-spermatogonia, primary and secondary spermiocytes) is through the application of cytogenetic methods. Now that suitable cytogenetic techniques for the study of the seminiferous epithelium of bulls have been developed (*Melander & Knudsen* 1953, *Knudsen* 1954, *Postiglioni-Grimaldi* 1956, 1957), the way is clear to re-examine the question of the types of testicular hypoplasia with differentiation of the seminiferous epithelium in bulls.

Lagerlöf (1948) has described a hereditary testicular hypoplasia in Holstein-Friesian bulls in which spermiogonia, spermiocytes, and even spermids are found but few or no sperms. A preliminary report dealing with the cytogenetic observations in such testicles was published (*Knudsen* 1958), and it was mentioned that sticky chromosomes were the cause of the suppressed development of the seminiferous epithelium of some of these bulls. The cytological observations on bulls with testicular hypoplasia caused by sticky chromosomes will be described in detail in this paper and placed in relationship to the other clinical findings. For this reason, only bulls which were available for thorough clinical and cytological study are included.

MATERIAL

Six bulls met these requirements. Five of these were from highly inbred Holstein-Friesian lines and one bull was of the Swedish Red-and-White breed. The ages of these bulls ranged from one to three years, i. e. they were examined before being used for breeding or as soon as it became apparent that they were sterile. According to the owners all these bulls had been used on fertile cows but none had sired offspring.

METHODS

The bulls were maintained in the clinic for three to six months; in the case of one animal, for only one month. During this time repeated evaluations of their general clinical status were made including at least five blood counts. Special interest, of course, was paid to the sexual organs.

At least ten ejaculates from each bull were collected in an artificial vagina. The volume of each ejaculate was measured. If the ejaculate contained sperm or sperm-like structures the concentration of these was estimated in a Bürkerchamber. In all instances the semen was centrifugated. Smears of the sediment were made before and after fixation in acetic alkohol (1:3). The smears were stained with eosin and Mayer's haematoxylin or with Gomori's haematoxylin.

For cytogenetic studies testicular tissue was obtained by castration or after slaughter and fixed in acetic alcohol (1:3). Squash preparations were stained with Gomari's haematoxylin in *Melander & Wingstrand*'s (1953) modification and histological sections with Gomori's haematoxylin and fast green (*Knudsen* 1959).

RESULTS

General clinical examination. No signs of non-sexual disease were demonstrated. All these bulls displayed strong libido and served eagerly and rapidly with good thrust. There was slight bilateral testicular hypoplasia in all the bulls, but no detectable differences in the size of the two testicles.

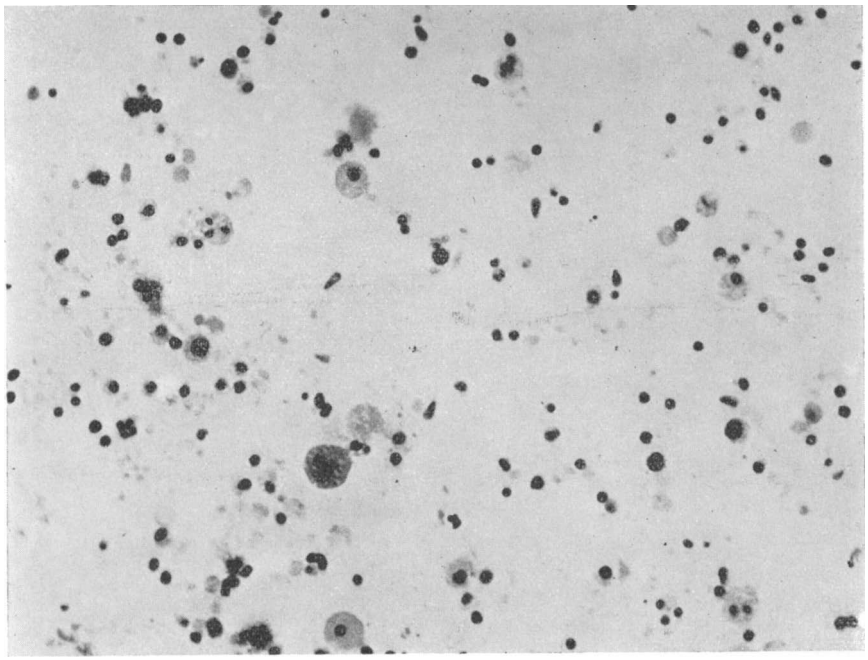

Fig. 1. Ejaculate sediment from a bull with testicular hypoplasia caused by sticky chromosomes. (Smear, approx 320 ×).

Ejaculate. The ejaculates were watery; aspermia or oligospermia were demonstrated. In the sediment it was unusual to find cellular elements other than pyknotic nuclei (Fig. 1). Sometimes these nuclei were enclosed in a drop of cytoplasm to give the appearance of a polynuclear giant cell. The pyknotic nuclei and the absence of a definite cell membrane serve to distinguish these structures from real cells. If sperm or sperm-like structures were encountered in the ejaculate, these were hyperchromatic and deformed often in the manner illustrated in Figs. 5 & 6. The greatest number of these bodies observed was 72,000/mm.^{3}, seen in the first ejaculate taken from the Red-and-White bull. Subsequent ejaculates obtained on the same occasion contained

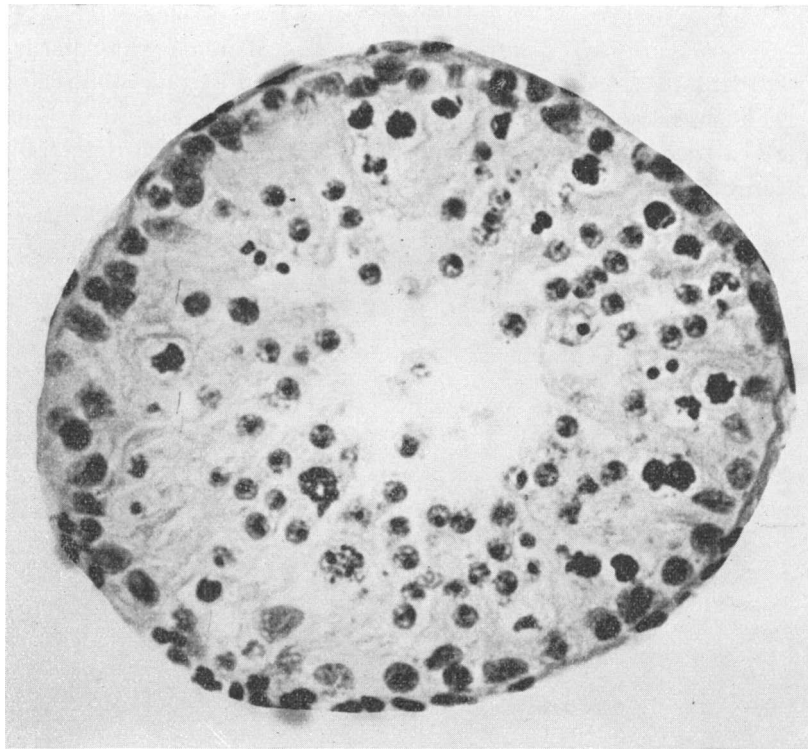

Fig. 2. Seminiferous tubule with pyknotic nuclei scattered diffusely throughout the epithelium. (Section, approx 360 ×).

26,000/mm.^{3} and 21,000/mm.^{3} respectively. On later occasions the number of these bodies was much reduced, 7,000/mm.^{3} at the most. Among the Holstein-Friesian bulls they were much fewer, 1,700/mm.^{3} were the most seen on any occasion. From these animals as well, the greatest numbers were encountered in the first ejaculates taken. If the bulls served often, the numbers of pyknotic nuclei and sperm-like bodies were greatly reduced only to increase again after a period of sexual rest. No other quantitative alterations in the ejaculates have been observed.

Morphological observations. Testicular weights for these bulls ranged between 180 and 280 g. The normal testicular weight for bulls of the same group of age and breeds is approximately 450 g. (*Lagerlöf,* 1934, 1948). The consistency of the testicles was normal but the cut surface was somewhat more glistening than in usual.

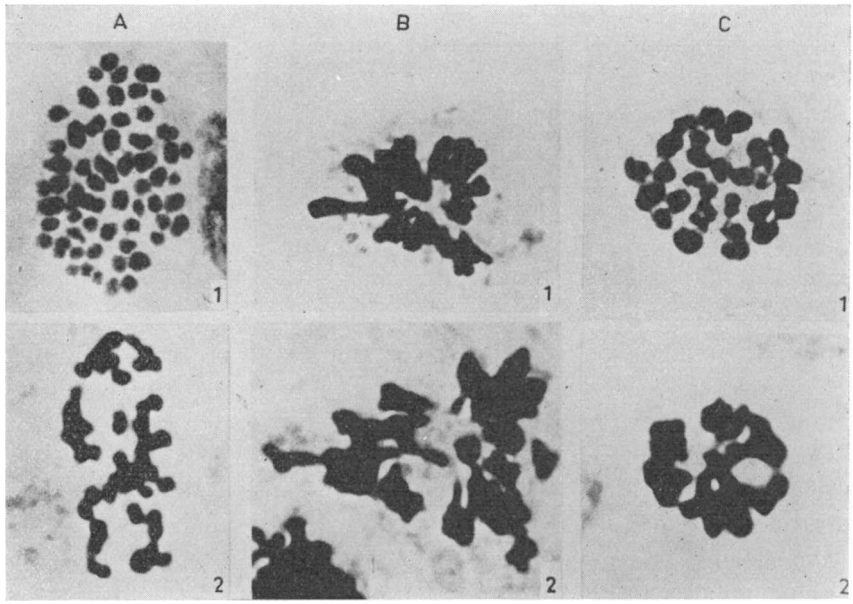

Fig. 3. Squash preparations showing various stages of spermiogenesis. The upper row represents normal spermiogenesis (1) and the lower row spermiogenesis in a bull with testicular hypoplasia caused by sticky chromosomes (2). (Squash, approx 1800 ×).
A. Metaphase of a B-spermiogonium. B. Diakinesis. C. Metaphase of a primary spermiocyte.

The cellular contents in the seminiferous tubules of these bulls are remarkably uniform both quantitatively and qualitatively. Empty tubules were encountered only occasionally. Since there are no adequate methods available, a statistical evaluation of this feature has not been made. The cell contents in the majority of the tubules occupy about one third of the radius from the basal membrane towards the centre. The free border towards the lumen is poorly defined since the cells here are more or less detached from the rest of the seminiferous epithelium (Fig. 2). Pyknotic nuclei are fairly uniformly distributed throughout the epithelium. Unlike the severe type of acute testicular degeneration (*Knudsen* 1954) in which the pyknotic nuclei are largely found in the centre of the seminiferous tubules, in bulls with sticky chromosomes pyknotic nuclei are present in all layers from the spermiogonia and inwards.

Cytological studies. Cell divisions among the A and B spermiogonia follow more or less a normal course even if one can

occasionally spot signs of chromosomal stickiness. During mitotic divisions in normal bull testicles, the chromosomes appear as solitary bodies (Fig. 3 A:1) while in the bulls studied here, the chromosomes stick together (Fig. 3 A:2).

During the first stages of division of the primary spermiocyte, the interphase and the leptotene stage, signs of disturbance in chromosomal behaviour are seen only occasionally. The chromosomes are still univalent at this period. With progression through subsequent stages — zygotene, pachytene, diplotene, and diakinesis (Fig. 3 B:1) — the chromosomes become arranged in pairs to give bivalents and form chiasmata and at the same time, they are concentrated into a bouquet arrangement within the nucleus (*Knudsen & Bryne* 1960). The complicated behaviour of the tightly-packed chromosomes during these later stages magnifies the effect of chromosomal stickiness upon the cells. This is most readily apparent in the failure of synchronisation of chromosomal development within the cells. Pachytene cells with a normal appearance, however, can be seen in moderate numbers. Later stages of the primary spermiocytes with uniform development of the chromosomes are quite rare. Some of the chromosomes in such cells remain in a stage resembling the pachytene while others can give the impression of the diplotene stage or are morphologically equivalent to metaphase chromosomes. In addition, complicated abnormal bivalents or multivalents are often encountered at the same time (Fig. 3 B:2). It is quite probably these changes making orientation toward the equatorial plane difficult which account for the often widespread scattering of the chromosomes along the spindle. In the normal bull, both chromosomes in each bivalent are closely applied to each other during the metaphase of the primary spermiocyte (Fig. 3 C:1). The various bivalent pairs are also tightly packed together during this stage (Knudsen & Bryne). Because of their stickiness, the two chromosomes do not separate in the customary manner as the anaphase commences, but instead become pyknotic. It has often been noticed that nuclei in which all chromosomes have reached the equatorial plane are undergoing pyknosis (Fig. 3 C:2).

Early anaphase configurations occur in relatively great numbers in these testicles. In such cells there are invariably several bridges between the two chromosome groups. And in the few more advanced anaphase configurations which were found among

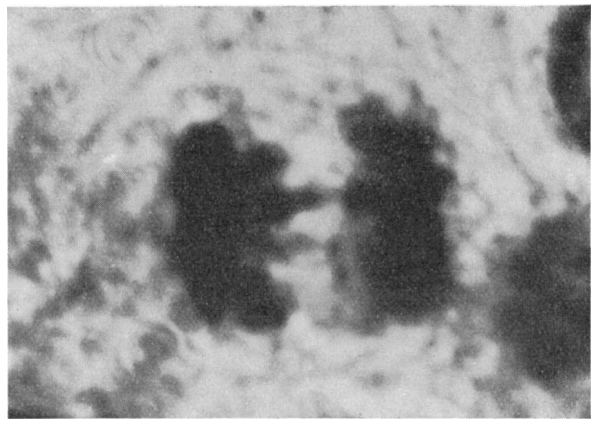

Fig. 4. Anaphase with bridges and loose fragments due to sticky chromosomes in a primary spermiocyte. (Section, approx 5000 ×).

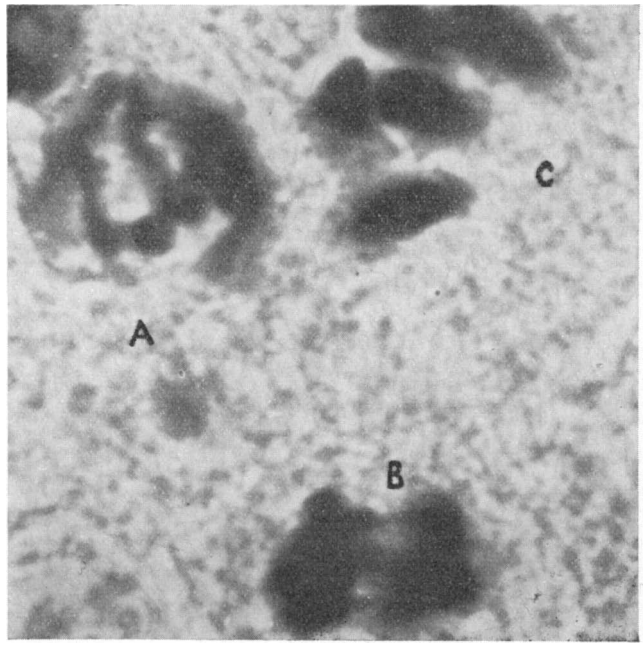

Fig. 5. A. Sticky chromosomes of non-uniform morphology in prophase of a secondary spermiocyte. B. Bridge formation between the two chromosome groups in anaphase of a secondary spermiocyte. C. Hyperchromatic spermids. (Section, approx 3500 ×).

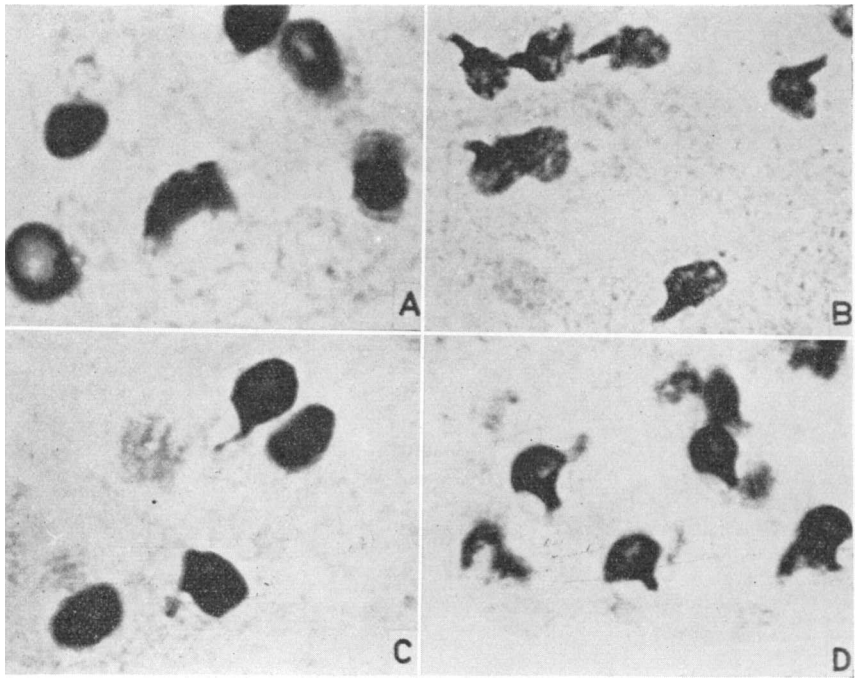

Fig. 6. Different types of pathological spermids resulting from sticky chromosomes. (A & D section, B & C squash, approx 2000 ×).

the primary spermiocytes, there were numerous fragments and in some instances even bridges of varying appearances between the chromosome groups (Fig. 4).

Secondary spermiocytes, or at least cells which resemble them, are found in moderate numbers. As in normal bulls these cells have a distinct nuclear membrane and the chromosomes are evenly distributed. In some prophase cells the chromosomes are morphologically fairly uniform although they are quite unlike the chromosomes of normal secondary spermiocytes. It is more usual, however, to find wide variations in chromosome morphology within the individual prophase cells. Many of the chromosomes are fused together in a chromatin drop or irregular body (Fig. 5 A).

Few metaphase stages are encountered among the secondary spermiocytes. Here as well chromosome morphology varies greatly. Most of these cells contain one or several large or small chromatin clumps which are not arranged along the metaphase plate. In the few anaphase configurations, the chromosomes are

severely deformed with great individual variations in size and shape and in early anaphase many bridges are present (Fig. 5 B).

The morphology of the centrosomes and spindles in all stages of spermiogenesis do not deviate from the appearance seen in normal bulls. Degenerative changes of the centrosomes and nuclear spindles of the type appearing in acquired disturbances of spermiogenesis are not observed in the bulls with sticky chromosomes. In these bulls, in fact, it is quite usual for the chromosome configuration to collapse while the spindle and centrosomes still maintain their morphological integrity.

Cells resembling spermids but of widely varying morphology are often encountered in large numbers. Although a hasty glance under low magnification can give the impression that some tubules contain normal spermids, closer examination invariably reveals the extreme variations in size, shape, and density of the cells. In the early stages of spermid formation groups of hyperchromatic and hypochromatic cells and even groups of cells of normal size and density but quite abnormal in form can be encountered in a single tubule. Among older spermids which have escaped pyknosis these morphological peculiarities are even more evident. Some are kidney-shaped and hyperchromatic (Fig. 5 C), others resemble open umbrellas (Fig. 6 D), still others tadpoles or sperm and so on (Fig. 6 A, B, C). In smears, groups made up of 4 or 8 of these sperm-like bodies are conspicuous. In bulls with aspermia all these cells end up in karyolysis or karyopyknosis; oligospermia implies that a few of these groups happen to differentiate into sperm. There are, then, degrees of severity in this particular abnormality in spermiogenesis in different bulls.

DISCUSSION

The low testicular weights and the unequivocally defective development of the seminiferous epithelium form the basis for the diagnosis testicular hypoplasia.

The morphology of cell divisions prior to the pachytene stage of the primary spermiocytes does not remarkably deviate from the normal. The few cells of the mitotic series (A and B spermiogonia) in metaphase which were pyknotic are per se relatively unimportant but do give a hint of chromosomal stickiness. Chromosome morphology during cell divisions succeeding the pachytene stage can only be described as chaotic. During the first meiotic division (primary spermiocyte), metaphases with a

single chromatin mass are common, chiasma formation is abnormal, abnormal and complicated bivalents and multivalents abound, the dynamic progression of the process within the cell is hindered, and numerous fragments appear even in the metaphase. All this is characteristic for stickiness (*Johnsson* 1948). In normal spermiogenesis, bivalents with a chiasma situated close to the centromere end often appear to have difficulty in orientation toward the equatorial plane (*Melander & Knudsen* 1953). It is quite clear, then, that congression will be more difficult if stickiness interferes with the formation of bivalents and chiasmata. Difficulties in co-orientation account for the feature so characteristic for these bulls, the lack of synchronisation in the development of the chromosomes during the first meiotic division. In cattle, the morphology of the bivalents from pachytene to metaphase depends to a great degree upon dynamics within the cell (Knudsen & Bryne). Bivalents with delayed or suppressed co-orientation are, morphologically at least, in an earlier stage of development than bivalents which have established co-orientation.

Early anaphase stages are very rarely encountered in specimens from normal bulls. The great number of nuclei in this stage in specimens from bulls with sticky chromosomes is probably an expression of the adhesion of the two chromosome groups by the numerous bridges. The rarity with which bridges are encountered in the later stages of anaphase probably results from the exceptionally short chromosomes of the bull being unable to form long bridges as easily as longer chromosomes. Late anaphase is dominated instead by the numerous fragments.

Morphological variations in the secondary spermiocytes and spermids must be considered sequelae to the disturbances in the cell division of earlier stages. The tendency for morphologically similar cells to form groups demonstrates that the cell division in some instances can proceed in spite of the stickiness but that the distribution of chromosomes among the daughter cells can be haphazard.

The greatly deformed cells of the later stages of spermiogenesis generally undergo karyolysis or karyopyknosis. The visible result in the ejaculate is the dominance of pyknotic nuclei, so characteristic that this gives presumptive evidence for the diagnosis of stickiness. With less severe degrees of chromosomal stickiness, sperm or sperm-like bodies can be formed. These

structures are highly chromatic and unlike the pathological sperm seen in acquired disturbances in spermiogenesis.

Toxic substances of various types are well known as a cause of sticky chromosomes in plants. Judging from writings on the subject, it is uncertain whether or not sticky chromosomes in mammals can result from intoxication. When a mammal is exposed to the effect of toxic substances, the metabolism of the body is likely interfered with in a different manner and degenerative changes would appear in the seminiferous epithelium long before stickiness could be expected (*Vlahos et al* 1954). It is unlikely that sticky chromosomes are produced through the influence of an external agent. The bulls discussed here had been kept under careful clinical observation for a sufficiently long time to dismiss intoxication as a factor in aetiology.

It was in 1932 that *Beadle* first demonstrated a gene for sticky chromosomes as a cause of sterility in maize. Nowadays stickiness is well recognized as a cause of sterility in plants. In mammals, on the other hand, sticky chromosomes have not previously been demonstrated as a cause of sterility. With the limited number of bulls available for this study, it has not proved possible to incriminate a particular gene as the cause of the defect. One of the bulls belonged to the Swedish Red-and-White breed, the others were Holstein-Friesians. In addition to these 6 bulls, sticky chromosomes have been demonstrated in some other bulls but they were not available for thorough clinical and cytological studies and accordingly have not been included in this report. Detection of this defect in various lines and different breeds does not necessarily contradict the hypothesis of a genetic background for the aberration in spermiogenesis. On the contrary, these is reason to suspect that the defect is a general biological phenomenon and that it can be expected in several breeds.

The changes in the seminiferous epithelium were uniform with practically total aspermia in the Holstein-Friesian bulls and a somewhat milder degree of stickiness in the Red-and-White bull. For the diagnosis of these milder degrees, not only the pyknotic nuclei but also the hyperchromatic sperm and sperm-like bodies in the ejaculate should be taken into account. *Maroulis* (1956) has mentioned karyopyknosis of human sperm but his description does not fully tally with the appearances seen in the bulls with extreme stickiness. From available knowledge on the mechanism of pyknosis (*Leuchtenberger,* 1949) it would appear

that milder degrees of stickiness could give nuclear pyknosis of the type described by Maroulis.

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SUMMARY

Sticky chromosomes have been demonstrated in the seminiferous epithelium of 5 Holstein-Friesian bulls and one Swedish Red-and-White bull, all sterile, with bilateral testicular hypoplasia and aspermia or oligospermia. Characteristically, the centrifugated ejaculate sediment was dominated by pyknotic nuclei and to a lesser extent, hyperchromatic sperm-like bodies.

In histological sections, pyknotic nuclei were evenly distributed throughout the seminiferous epithelium. All seminiferous tubules within a given testicle were affected to much the same degree.

Stickiness of the chromosomes in cell divisions preceding the primary spermiocytes is quite probably of little or no importance. When the chromosomes form bivalents in the primary spermiocytes,

the course of cell division is interfered with to such a degree that morphologically the succeeding stages can be described as chaotic. Typically, primary spermiocytes are encountered with chromosomes in various stages of development and with abnormal or complicated bivalents and multivalents, free chromatin fragments, or with all the chromosomes gathered into a single chromatin mass. In early anaphase configurations, there are often bridges while in later anaphase and in telophase, the chromosomal groups are often disintegrated into fragments. Among secondary spermiocytes and spermids, there is a tendency for cells of a given morphological type to form groups. The morphology of these cells varies widely from group to group but the histological appearance is dominated by karyolysis or karyopyknosis. Cells of the latter type, of course, are the origin of the pyknotic nuclei seen in the ejaculate.

All the bulls described in this paper showed severe chromosome stickiness. It is likely, however, that the defect can occur in various degrees. Milder forms ought to be clinically manifest through the pyknotic nuclei and sperms with hyperchromatic nuclei in the ejaculate.

ZUSAMMENFASSUNG

Klebrige Chromosomen (Stickiness) als Ursache zur Hoden-Hypoplasie bei Bullen.

Bei fünf Bullen der Schwedische Niederungsrasse und einem Bullen der Schwedische Rot- und Weiss-Rasse mit bilateraler Testikelhypoplasie war Stickiness (klebrige Chromosomen) die Sterilitätsursache. Die Bullen haben Aspermie oder Oligospermie. Das Spermacentrifugat ist durch pyknotische Kerne, stark kromatische, spermienähnliche Körper karakterisiert.

Das histologische Bild weist gleichmässige Verteilung von pyknotischen Kernen in den Tubuli seminiferi über den ganzen Testikel auf.

In den Zellteilungsstadien vor den primären Spermiozyten hat die Klebrigkeit der Chromosomen geringe oder wahrscheinlich keine Einwirkung. Wenn die Chromosomen Bivalenten in den primären Spermiozyten gebildet haben, wird der Zellteilungsverlauf so gestört, dass die Morphologie in den folgenden Stadien geradezu als chaotisch bezeichnet werden kann. Typisch sind primäre Spermiozyten mit Chromosomen in verschiedenen Entwicklungsstadien, anormale und komplizierte Bivalenten und Multivalenten, lose Chromosomfragmenten oder das ganze Chromosomkomplement in einer einfachen Chromatinmasse gesammelt. Früh in der Anaphase sieht man Brücken, während im Bild der späteren Anaphase und Telophase Fragmentbildungen vorherrschen. Sekundäre Spermiozyten und Spermiden haben eine Tendenz zur Gruppenbildung von morphologisch gleichartigen Zellen. Die Morphologie variiert in den verschiedenen Gruppen, wird aber von Karyopyknosis oder Kariolysis beherrscht, was sich auch im Ejakulat in Form von pyknotischen Kernen zeigt.

Die hier beschriebenen Bullen haben alle hochgradige Stickiness. Der Verfasser ist jedoch der Ansicht, dass die Anomalien in ver-

schiedenen Schwierigkeitsgraden vorkommen können. Leichtere Formen zeigen sich dann wahrscheinlich klinisch im Ejakulat in Form von pyknotischen Kernen oder Spermien mit chromatischen Nucleus.

SAMMANFATTNING

Klibbiga kromosomer (stickiness) som orsak till testikelhypoplasi hos tjur.

Hos 5 tjurar av SLB-ras och en tjur av SRB-ras med bilateral testikelhypoplasi har stickiness (klibbiga kromosomer) påvisats som sterilitetsorsak. Tjurarna ha aspermi eller oligospermi. Spermacentrifugatet karakteriseras av pyknotiska kärnor och eventuellt av starkt kromatiska spermieliknande kroppar.

Den histologiska bilden visar jämn fördelning av pyknotiska kärnor i Tubuli seminiferi över hela testikeln.

På celldelningsstadierna före de primära spermiocyterna har kromosomernas klibbighet ringa eller sannolikt ingen inverkan. När kromosomerna bildat bivalenter i de primära spermiocyterna rubbas celldelningsförloppet till den grad att morfologin i efterföljande stadier närmast kan betecknas som kaotisk. Typiskt är primära spermiocyter i olika utvecklingsstadier, onormala och komplicerade bivalenter och multivalenter, lösa kromosomfragment eller hela kromosomkomplementet samlat i en enkel kromatinmassa. I tidig anafas ses oftast bryggor medan i senare anafasstadier och telofas fragmentbildningar dominera bilden. Sekundära spermiocyter och spermider har en tendens till gruppbildning av morfologiskt likartade celler. Morfologin varierar avsevärt i olika grupper men domineras av karyopyknosis eller karyolysis, vilket också visar sig i ejakulatet i form av pyknotiska kärnor.

De här beskrivna tjurarna ha alla höggradig stickiness, men författaren anser att anomalien kan förekomma i olika svårighetsgrad. Lindrigare former bör då kliniskt visa sig i ejakulatet i form av pyknotiska kärnor och spermier med hyperkromatisk nucleus.

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