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Germ Cell Weakness as a Cause of Testicular Hypoplasia in Bulls

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Settergren, I. and K. McEntee.: Germ cell weakness as a cause of testicular hypoplasia in bulls. Acta vet. scand. 1992, 273-282. – Sporadic cases of testicular hypoplasia were earlier found in bulls of the Swedish Red and White breed. An accumulation of cases have occurred since 1970 in sons of 2 outstanding progenytested bull sires, 2 F and 27 U, which had a common father, 545 B. The history and clinical examination of affected bulls varied. Some had azoospermia and very small testes at a young age, while others could be normal in all respect when they were young but had a short reproductive life and had to be culled at about 3 years of age. Most of the affected bulls were between these 2 extremes.

The histologic examination showed principally different degrees of testicular degeneration. There were always some germ cells left in all affected seminiferous tubules indicating that there was not a lack of germ cells causing the hypoplasia.

Germ cell weakness is obviously a hereditary condition. The sires 545 B, 2 F and 27 U had a relatively low fertility. In their pedigree were several bulls known to have had a low fertility. No sons of 2 F and only a few sons of 27 U were used for A.I. services and at present only few cases of testicular hypoplasia are seen.

hereditary; histology; clinical symptoms.

Introduction

Testicular hypoplasia is a congenital and generally hereditary condition, which can be unilateral or bilateral and total or partial. Further the testes are usually smaller than normal and produce relatively few sperm cells of inferior quality.

Testicular hypoplasia in the Swedish Highland breed (SKB) was described extensively by *Lagerlöf* (1934, 1938, 1951) and *Eriksson* (1943). Lagerlöf showed that the hypoplasia was usually leftsided (about 88%) but could also be rightsided (about 4%) or bilateral (about 8%). It could further be partial or total. Histological investigations showed that there were no germ cells in the whole testicle or in part of it. The genetic analysis by *Eriksson* (1943) revealed that the hypoplasia was caused by a recessive gene with incomplete penetrance. Later investigations by *Settergren* (1961) showed that there was a very significant relationship between hypoplasia and white colour of the body and ears.

Lagerlöf (1934) also reported another type of testicular hypoplasia in the Swedish Red and White breed (SRB). The testes were small, 200-300 g in an adult bull, the number of sperm cells was low, 50,000-150,000/mm^3 and the quality of the sperm cells was inferior. Varying numbers of the seminiferous tubules were affected and showed a picture resembling testicular degeneration. As there were few cases over the following years no further

investigations were undertaken and the possible hereditary background has not been analyzed.

However, during the last 20 years a number of cases of this type of testicular hypoplasia was found, especially among the sons of 2 SRB bull sires that were half-brothers. *Lundgren* (1972) gave a preliminary report on some of the bulls listed in Table 1 but did not mention anything about the histology.

The aims of the present study are to describe the history, clinical findings, semen quality and histology in cases of germ cell weakness.

Material and methods

The material consists of 41 bulls from 5 different A.I. stations (Tables 1 and 2). Twentyone bulls were the sons of the sire 2 F and 20 sons of the sire 27 U. They were all raised at the rearing stations used by the A.I. associations from the age of 6-8 weeks until they were 1 year old. At the stations the bulls were kept under standardized conditions and were fed according to the growth rate. After the growth test most of the bulls that were below the average curve for the growth rate were culled, while the rest of the bulls underwent tests for fertility, comprising serving ability, semen quality and freezability. The semen of those bulls which had passed both tests was used for test inseminations. All bulls were karyotyped in connection with the growth test. Eleven of the bulls, 6 sons of the sire 2 F and 5 sons of the sire 27 U, were brought to the clinic of the Department of Obstetrics and Gynaecology of the Veterinary College for further investigations when it was decided to cull them. They were kept here for periods of 1 to 8 months, most of them for about 2 months.

At the time of slaughter all bulls were brought to a slaughterhouse, where they were killed and treated according to Swedish rules for slaughterhouses. The organs were examined within 2 h of slaughter.

The testes were inspected, measured and weighed. Then each testis was cut in 2 halves along the mediastinum. Each half was then cut into slices about 1 cm thick and carefully examined for macroscopical lesions. Small pieces were taken for histology from the proximal and distal parts of each testis and immediately fixed in Bouin's solution. After processing and embedding in histowax the tissue was cut in 5 μm thick sections. Some sections were stained with hemalum-eosin and some with van Gieson stain.

The epididymides as well as the accessory sexual organs and penis were inspected for macroscopical abnormalities and if needed pieces of the tissues were taken for histology. Content from both epididymides was fixed in buffered formalin and the sperm cell morphology examined.

Results

History and clinical examination

Of the 21 bulls in Table 1 two were culled because of low growth rate and 4 because enough semen had been collected from them. The remaining 15 bulls were culled because of poor semen quality or low fertility. Of 20 bulls in Table 2 eleven were slaughtered because of low growth rate, while for the other 9 bulls the reasons were poor semen quality or low fertility.

Bulls with poor semen quality or low fertility at the time of culling had a varied history. A few had azoospermia at 1 year of age or a very low sperm cell concentration combined with small testes and a poor semen picture. Other bulls had normal semen, freezability of sperm cells and fertility when they were first tested but the fertility or semen quality declined from the age of 20-30 months. The remaining bulls were between these 2

Tabel 1. Demographic data for sons of sire no. 2 F.

Bull no.	Age at slaughter (months)	Testis weight (g)		Reason for culling	Semen quality	Testicular histology
		Left	Right			
959 M	13	191	218	Low growth rate	Not examined	c
4 A	13	143	162	Low growth rate	Not examined	c
945 M	15	192	216	Poor freezability	Low conc. loose heads	a
118 S	16	207	228	Poor freezability	Low conc.	a
210 B	16	187	189	Semen quality	Low conc., poor motil.	c
5 J	16	202	198	Semen quality	Very low conc., poor motil.	c
414 D	18	234	330	L test. small, low fertility	Fairly normal	c
356 K	21	261	242	Low fertility	Normal	c
24 H	21	230	240	Low fertility	Low conc., poor motil.	c
648 b	21	218	250	Low fertility	Normal	a
711 B	22	217	217	Poor freezability	Fairly normal	c
888 B	22	220	246	Low fertility	Fairly normal	b
194 S	23 (16)	(144)	185	Hypoplasia	Aspermia	b
483 H	31	Left	smaller	Low fertility	Varying, low motil., loose heads	b
59 S	33	308	311	Enough semen	Decreasing	a
304 F	33	404	415	Enough semen	Normal	a
347 R	34	318	356	Enough semen	Normal	a
882 B	35	280	331	Semen quality	Decreasing	b
523 B	35	189	268	Poor freezability	Decreasing	a(R) c(L)
12 b	36	413	408	Poor freezability	Decreasing	a
36 T	39	308	331	Enough semen	Good	a

a = mild, b = moderate, c = severe degeneration. R = right, L = left.

Tabel 2. Demographic data for sons of sire no. 2 F.

Bull no.	Age at slaughter (months)	Testis weight (g)		Reason for culling	Semen quality	Testicular histology
		Left	Right			
32 M	13	165	160	Low growth rate	E. s. = tail changes	a
395 S	13	195	214	Low growth rate	E. s. = unripe	b
201 M	13	175	176	Low growth rate	E. s. = low conc., unripe	b
244 S	14	230	230	Low growth rate	E. s. = tail changes, unripe	c
8 S	14	176	191	Low growth rate	E. s. = loose heads, unripe	b
653 Å	14	–	–	Low growth rate	E. s. = tail changes, unripe	c
18 B	14	256	258	Low growth rate	E. s. = fairly normal	a
15 H	14	225	247	Low growth rate	Not tested	b
37 B	14	227	218	Low growth rate	E. s. = loose heads, unripe	b
127 S	15	263	269	Low growth rate	E. s. = tail changes left	b
1102 S	15	24	196	L kryptorchid	Does not serve	b (R)
226 S	15	265	278	Low freezability	Fairly normal	c
69 L	15	209	220	Low growth rate	E. s. = tail changes, unripe	b
140 F	16	218	188	R semi-kryptorchid	Tail changes, detached heads	c
72 S	17	219	201	Poor serving ability	E. s. = fairly normal	b
120 B	17	255	251	Low freezability	Not tested	a
218 K	18	209	219	Low sperm conc., small testes	Low conc. and motil., detached heads	a
40 H	19	246	255	Low sperm conc. and freezability	Low conc., poor morphology	a
162 B	20	284	297	Low freezability and fertility	Conc. varying, motil. going down	b
154 Ö	20	263	280	Low fertility	Slow maturation	b

a = mild, b = moderate, c = severe degeneration. R = right, L = left, E. s. = epididymal sperm. mos =

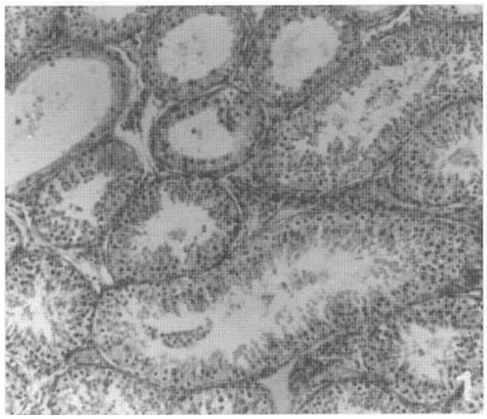


Fig. 1: Germ cell weakness with mild testicular lesions. 80 ×.

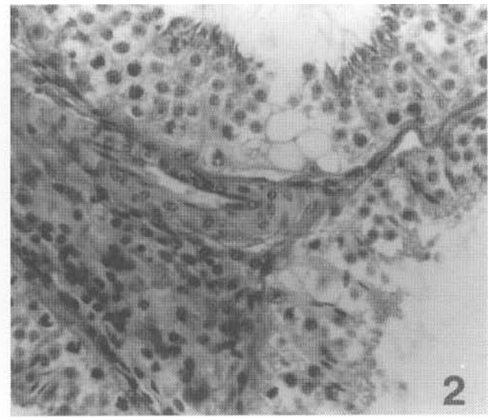


Fig. 2: Vacuoles in the germinal epithelium. 240 ×.

extremes. Some bulls for instance had normal semen picture and freezability but had a low fertility.

The 4 bulls in Table 1 which were culled because enough semen had been collected from them, had normal semen and fertility when they were young and in 3 of them the semen and freezability were still normal at time of culling. However, because of the risk of transmitting the predisposition for germ cell weakness, their semen was not used for A.I.

Eleven of the bulls in Table 2 were slaughtered because of low growth rate. Their semen was not tested but in 10 of them epididymal sperm cells were examined for morphology. Nine of the 10 bulls had abnormal sperm morphology.

The clinical examination of the bulls usually showed that the testes were small in those bulls where the semen picture deviated from the normal and the concentration of sperm cells was low. The testes were soft at superficial palpation and relatively firm at deeper palpation.

A difference in weight between the 2 testes of more than 10 % can be regarded as outside the normal variation and is possible to establish at palpation. According to this criterion, in 9 of the 20 sons of the sire 2 F (Table 1) with known testicular weight at the time of slaughter the left testes were smaller than the right. If the two kryptorchid bulls are removed none of the remaining 17 sons of the sire 27 U (Table 2) with known testicular weights had different size of the testes.

Histopathology

The testicular lesions were principally the same as in testicular degeneration. They were classified into 3 groups (mild, moderate and severe) according to the degeneration of the seminiferous tubules.

The mild lesions occurred in testes in which the vast majority of seminiferous tubules appeared to be normal. There were a very few areas in which the tubules were markedly deficient in germ cells. The Sertoli cells appeared to predominate but there were always a few germ cells present. Usually there were not more than 3 to 5 cross sections of a deficient tubule in any one area

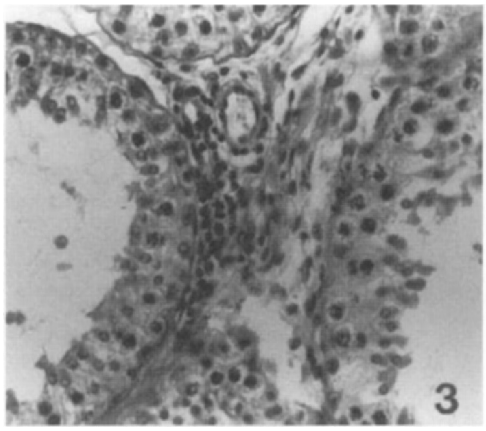


Fig. 3: Perivascular accumulation of lymphocytes in testicular interstitium. 240 ×.

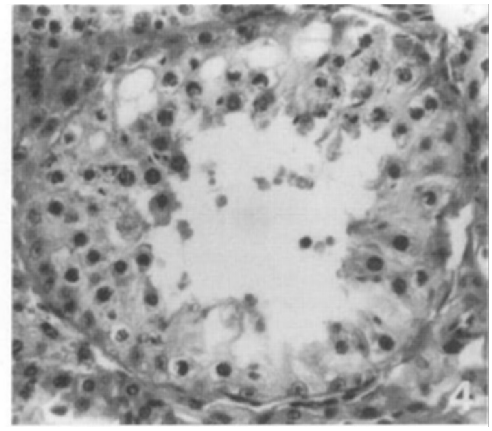


Fig. 4: Tubule with degenerating spermatocytes and spermatids, vacuole formation. 240 ×.

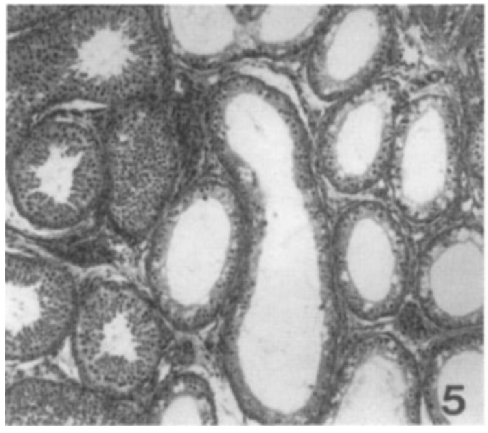


Fig. 5: Germ cell weakness with moderate testicular lesions. 60 ×.

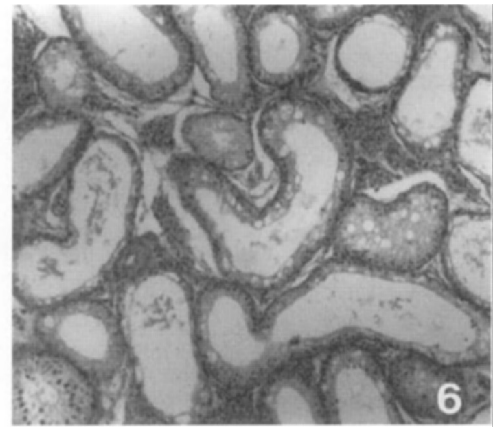
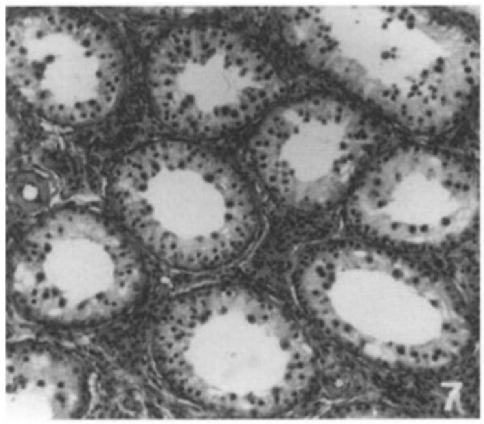


Fig. 6: Germ cell weakness with severe testicular lesions. 60 ×.

(Fig. 1). There were a few other tubules containing pycnotic primary spermatocytes and spermatids but the majority of pycnotic cells appeared to be primary spermatocytes. There were very few spermatid giant cells in the tubular lumina. Vacuoles, indicating loss of germ cells, were present in some tubules and occasionally adjacent to degenerating spermatocytes (Fig. 2). Some of the Sertoli cells had small masses of eosinophilic material in the cytoplasm. In some areas there appeared to be similar eosinophilic material in the nuclei of Sertoli cells but in other areas this could be recognized as an invagination of eosinophilic material from the cytoplasm. Some of the Sertoli cells were beginning to move away from the basement membrane toward the lumen.

A few perivascular accumulations of lymphocytes (Fig. 3), which sometimes extended



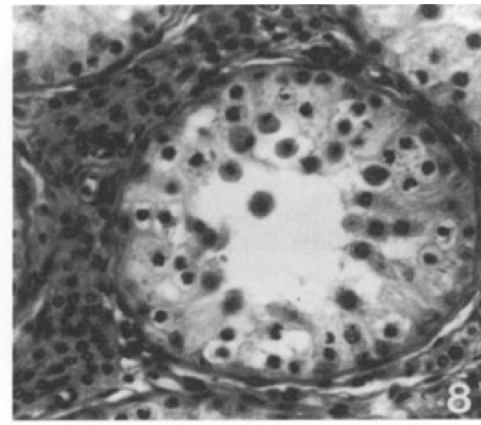


Fig 7 and 8: Widespread degeneration of the germinal epithelium. 100 × and 240 ×.

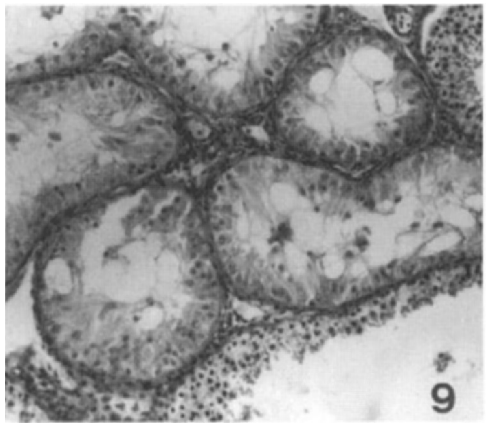


Fig. 9: Severe testicular lesions with migration of Sertoli cell nuclei. 100 ×.

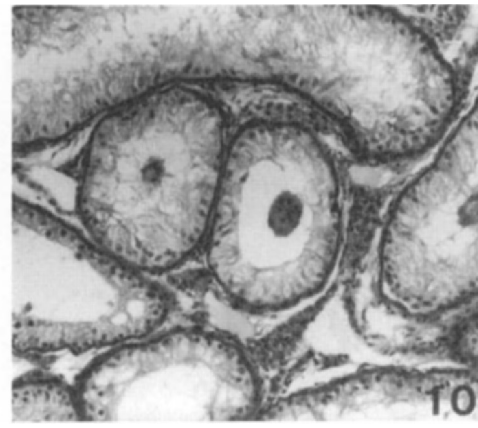


Fig. 10: Migrated Sertoli cell nuclei forming clusters in the centre of seminiferous tubules. 100 ×.

partially around a tubule in a crescent formation, were present in the interstitial tissue in most of the cases. A few plasma cells were also present in some cases. The inflammatory foci were usually adjacent to tubules with degenerating germ cells but were not found in all areas of degeneration.

In cases of moderate degeneration the most prominent feature was an increase in the number of seminiferous tubules with degenerating spermatocytes and spermatids and a marked increase of tubules with vacuole formation in the seminiferous epithelium (Fig. 4). There appeared to be an accelerated rate of degeneration of germ cells. In some cases, there was an increased number of tubules with advanced depletion of germ cells (Fig. 5). Perivascular accumulation of lymphocytes were present in all of the cases of moderate degeneration.

In the bulls with severe testicular degeneration, there were very large areas of degeneration of the seminiferous tubules (Figs. 6, 7, 8). The areas of degeneration often involved an entire tissue section measuring approximately 7 × 15 mm. The area of most severe degeneration was located most frequently in the dorsal portion of right testis. There was more advanced migration of Sertoli cell nuclei into the lumina and the cells located in the centre of the lumen were undergoing degeneration evidenced by shrinkage and increased eosinophilia of the cytoplasm (Fig. 9). These cells formed clusters in the lumina and were sometimes detached (Fig. 10). Perivascular accumulations of lymphocytes were present and appeared to be more common in the areas of early degeneration. They were not as prominent in areas where there was severe depletion of germ cells.

On the basis of histologic examination the Leydig cells did not appear to be abnormal in any of the bulls.

Discussion

As there is a large variation of symptoms in bulls with germ cell weakness it is necessary to have good information about the history including the pedigree, the findings from clinical examination of the sexual organs and semen as well as macroscopic and microscopic findings at the postmortem examination, to arrive at the right diagnosis. Typical for the history seems to be that affected bulls often mature rather late and that in those bulls which are fertile at a young age the reproductive live is short, usually not beyond 3 years of age. The testes are small but mostly of the same size. In the present material 9 of the 21 sons of the sire 2 F had left testes smaller than the right but in most of them the weight was still 85% or more of the weight of the right testis. The 20 sons of the sire 27 U had left and right testes of the same size.

The histological examination showed that the germinal epithelium in the seminiferous tubules was deficient but in all tubules there were at least some germ cells, even if they were degenerating. This is in agreement with what was found by *Lagerlöf* (1934) in some bulls of the Swedish Red and White breed (SRB). However, it is quite different from the testicular hypoplasia in the Swedish Highland breed (SKB) which is mostly unilateral and where the seminiferous tubules in the whole or part of 1 or both testes are completely devoid of germ cells (*Lagerlöf* 1934) and thus show a Sertoli cell-only type of hypoplasia.

Laing & Young (1956) described testicular hypoplasia in British cattle, which was more often leftsided than rightsided and seldom bilateral. The histology showed different degrees of deficiency, from a single layer of spermatogonia in the seminiferous tubules to a stage where a few sperm cells were formed.

Rao Veeramachaneni et al. (1986) found severe testicular lesions in 4 beef bulls with scrotal circumference below 30 cm. Some tubules had only Sertoli cells while in other there was a loss of germ cells and a vacuolated epithelium.

Krishnalingam et al. (1982) examined 7 cases of unilateral and 23 cases of bilateral testicular hypoplasia in young pure or crossbred Brahman bulls. The histology showed vacuolation of the germinal epithelium in all hypoplastic testes but also to a lesser extent in the normal ones. There was a migration of Sertoli cells to the lumen of the seminiferous tubules where they formed rings that looked like giant cells.

Markau (1963) found in a material of 225 Holstein-Friesian bulls 14 cases of total and 32 cases of partial testicular hypoplasia. In

total hypoplasia both testes were equally small and no sperm cells were produced. In about 90% of the seminiferous tubules there were only spermatogonia and spermatocytes. Partial hypoplasia could be unilateral or bilateral. In 40-70% of the seminiferous tubules there was an arrested spermatogenesis with no production of sperm cells.

In 35 inbred beef bulls *Carrol & Ball* (1970) found 10 cases of bilateral testicular hypoplasia. In 4 of them there was a complete hypoplasia of Sertoli cell-only type while the remaining showed different stages of testicular degeneration with formation of pseudogiant cells from spermatids.

In all the papers on testicular hypoplasia in bovine cited above there is some resemblance to germ cells weakness but nothing is mentioned about transitory fertility, where fertility at a young age is later followed by infertility and sterility. However, *Bruce* (1958) described genetic infertility in ruby-eyed male hamsters, which were fertile from puberty to 90 days of age, then had impaired fertility for another 60 days and were sterile after 150 days of age. The histology of affected testes looked very much like in testes with germ cell weakness.

Germ cell weakness obviously has a hereditary background but the exact nature has not been studied. The 2 sires of the examined bulls, 2 F and 27 U, have a common father, 545 B. This bull had 28 sons used for A.I. services, of which 13 were culled because of poor semen quality or low fertility. The sire 2 F was used for several years and his fertility was always 2-5 per cent units below the average for the A.I. centre. Also the sire 27 U had a fertility below average. In the pedigree of 545 B there are several bulls which are known to have had low fertility.

No one of the sons of the sire 2 F were used for A.I. in spite of a very high production of his daughters. Just a few of the sons of 27 U were used for A.I. and only 1 became a bull sire. By this tight control of the use of affected bulls there has not been any accumulation of new cases of hypoplasia caused by germ cell weakness. As the predisposition towards germ cell weakness is spread in the SRB breed it is, however, important to keep a continued good control of the fertility in the young AI-bulls.

Acknowledgements

The late Dr. Bengt Lundgren was the first one to recognize the problem with this type of hypoplasia. He and other colleagues at several A.I. centres have kindly supplied material for the investigations. Dr. Sven Viring was helpful in collecting information about the bull material which is gratefully acknowledged. The authors thank Ms. Annika Rikberg for excellent technical assistance and Ms. Marie Sundberg for preparing the manuscript.

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Sammanfattning

Svaghet i könscellerna som orsak till testikelhypoplasi hos tjur.

Sporadiska fall av testikelhypoplasi hos SRB-tjurar har varit känt sedan länge. Sedan 1970 har dock setts en anhopning av fall bland sönerna till de båda tjurfäderna 2 F and 27 U, vilka båda var avkomlingar till 545 U. Alla 3 tjurarna var relativt lågfertila. Anamnesen och den kliniska bilden hos defekta tjurar var mycket växlande. Några visade aspermi och mycket små testiklar redan vid de första spermasamlingarna. Andra kunde vara helt normal som unga och även ha normal fertilitet för en tid men visade snart en nedgång i fertilitet och spermakvalitet, så att de måste slås ut före 3 års ålder. Övriga tjurar visade symptom mellan dessa 2 ytterligheter. Histologiskt visade testiklarna en degenerationsbild. Även i höggradiga fall fanns dock alltid könsceller i tubuli seminiferi, även om de var degenererade. Detta visar att det rör sig om en principiellt annorlunda typ av hypoplasi än den som tidigare utförligt beskrivits för den svenska fjällrasen. I stamtavlorna för 545 B, 2 F och 27 U finns flera tjurfäder, som var lågfertila. Inga söner efter 2 F och endast få efter 27 U kom att användas i A.I. och som en följd av detta har någon ny anhopning av testikelhypoplasi inte förekommit, även om enstaka fall diagnosticeras.

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