TESTING SWISS BRAUNVIEH BULLS FOR THE INHERITED SYNDROME OF ARACHNOMELIA AND ARTHROGYROSIS (SAA) BY EXAMINING THEIR OFFSPRING AT THE FETAL STAGE

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SUMMARY
An inherited limb abnormality called syndrome of arachnomelia and arthrogryposis (SAA) has been observed in the Swiss Braunvieh population. A method is presented which allows detection of SAA carrier bulls by means of superovulation of known carrier cows, embryo transfer and examination of fetuses at about 100 days of gestation. A description of the deformity is given. The primary cause of the defect is attributed to an inherited neuromuscular dysfunction early in gestation. The segregation ratio confirms the hypothesis that this neuromuscular disorder is transmitted as an autosomal recessive trait with high penetrance. The use of this method in an AI program is outlined.

INTRODUCTION
The syndrome of arachnomelia and arthrogryposis (SAA) certainly occurred earlier in the Swiss Braunvieh population, but was recognized as a serious congenital defect only since the fall of 1984. Since then, numerous cases have been recorded, especially frequently in the second crop progeny of the then top A.I. proven bull.

Clinical findings
The deformity in these newborn calves involves both muscular and skeletal growth (Fig. 1). The legs are abnormally long and thin (arachnomelia or dolichostenomelia) with increased fragility of the long bones, especially of the metacarpi and metatarsi. Spontaneous fractures during parturition could result in injuries to uterus and genital passage. Ligaments, tendons and joints can be overstretched but also short or crooked (arthrogryposis of carpal, tarsal and fetlock joints). Skeletal muscles appear atrophied and hypoplastic. Changes on the nervous system (e.g. plexus brachialis) and other abnormalities such as brachynathia inferior, short skull with a dent in the frontal bone, kypho-skoliosis and possibly vessel aneurysms have also been observed. Most of the affected calves were stillborn or died during birth, only a few lived for a few hours. There is a great similarity with autosomal recessive syndrome of arthrogryposis and palatoschisis (SAP) in the Charolais breed (5, 6, 8, 9) and other reports (3, 7, 11). The same defect has been described in a German Braunvieh population as syndrome of arachnomelia (2).
Occurrence and inheritance

From November 1984 to October 1985, 297 cases of SAA were recorded in the registered population through the herdbook and the A.I. centers. This figure corresponds approximatively to 0.15% of the births in registered herds and is slightly lower than in the German report (2). Pedigree investigation of the affected calves very soon showed evidence for hereditary determination. The hypothesis of a single autosomal recessive gene fits all recorded cases and was confirmed through a survey on inbreed matings (10). According to preliminary estimations, the frequency of the recessive gene is about 2% in the cow population.

The origin of this recessive is attributed to an American Brown Swiss bull or to a cow of the same breed; both always appear in the paternal and in the maternal parts of the pedigrees. The SAA cases observed in Germany have been traced back to the same common ancestors (1). Semen from several offspring of these animals was widely used in Switzerland and had a very large influence on the breed.

Designing a test procedure

In order to reduce the incidence of the lethal defect, the first decision was to remove the known carrier bulls from A.I. service. In the following batches of waiting bulls, several bulls had carriers in their ancestry but had not themselves been revealed as carriers during sampling. The purpose of this study was to develop a reliable and fast method to test suspected bulls. Since no chromosomal or biochemical marker has been detected up to now, the only possibility is to mate the bull to be tested and screen his offspring. In order to save time a similar technique as applied in (4) to test syndactyly in Holstein-Friesian was considered, i.e. examination of fetuses.

High reliability is obtained at minimum cost in mating the test bull with known carrier cows instead of random sample of the population or daughters of known carriers. Table 1 shows the required number of offspring according to the desired error probability and the kind of matings. Using embryo transfer allows to reduce the number of cows involved in the program. Diagnosis on fetuses with the objective of minimizing time and costs of the test procedure is highly desirable.

Preliminary trial

The objective of the preliminary trial was to find out if diagnosis of SAA is possible on fetuses and, if yes, which is the earliest possible fetal stage for a safe diagnosis.

MATERIAL AND METHODS

Seven SAA carrier cows were superovulated and inseminated with one of two carrier bulls. The selected animals had previously all had typical SAA progeny. Flushed embryos were transferred nonsurgically in 24 recipient heifers. The conception rate was similar to that one in commercial programs. Recipients were slaughtered at different gestation length and the whole uteri were saved with the fetuses (1 empty, 15 singles, 16 twins). 31 fetuses were therefore available for examination.

Current investigations on age determination of bovine fetuses have demonstrated that normal ossification of the leg bones begins at about 60 days of gestation. Since the differences between normal and SAA fetuses could be assumed to become
detectable one to two months later, as demonstrated for arthrogryposis in the case of SAP (9), the trial started with 110 day old fetuses. The investigation was then extended to younger and older fetuses (Table 2) in order to assess differences at different stages of ossification.

RESULTS AND DISCUSSION

Careful examination of the material, including radiography, diagnosed 8 SAA positive animals, aged 116, 110, 105, 98 and 87 days (Table 2). Changes in the youngest group were barely perceivable.

Deformations observed in 8 fetuses with SAA

**Skull:** All fetuses had a 3-5 mm deep frontal dent just behind the sutura nasofrontalis (Fig. 2). In radiographs, the angle between the os frontalis and the palatum durum was of 75-85° (Fig. 4) instead of 50° as in normal animals. **Brachygnathia inferior** of moderate degree was found in six animals (Fig. 2).

**Vertebrae:** A distinct curvature of the spine mainly as a thoracolumbar kyphosis (Fig. 2) was present in all fetuses. In addition, one 116 day old fetus also had a kyphotic shoulder region and a marked reduction in height of all vertebrae (Figs. 3, 4).

**Leg bones:** The metacarpal and metatarsal diaphysis was extremely thin (35% decrease in diameter) in all fetuses. The diaphysial diameter of the other long bones was only slightly reduced. In the 116 day old fetus, the length of all fore- and hindleg bones, pelvic bones included, was also reduced (about 25-30%), whereas the epiphyses appeared normal or slightly widened (Figs. 3, 4).

**Forelimbs:** Due to a restricted joint movement of the carpi (Fig. 2), one or both forelegs of all fetuses could be extended only to an angle of about 100-130° (in two extreme cases only 70°) instead of 180° as in normal animals. The fetlocks were often maintained in slight flexion and movement was restricted.

**Hindlimbs:** The tarso-metatarsal region of six fetuses was slightly bent forward. Consequently the **plantar line** was convex (with an angle of 15-20°, (Figs. 2, 4) instead of concave as in normal animals. Due to a restricted joint movement of the genua and the tarsi, the hindlegs of the same animals could not be fully extended. In one fetus these joints and the fetlocks were extremely extended. The hindleg deformity was manifest in seven animals as a typical bilateral dorsiflexion of the fetlocks (to an angle of 30-50°, Figs. 2, 4). The convex plantar line and the dorsiflexion of the fetlocks correspond to the findings in SAP of Charolais cattle (9).

**Muscles:** The muscles of the neck, shoulder, lumbar and thigh region showed a padded contracture in all fetuses, especially in the older ones.

CONCLUSIONS

The preliminary trial was successfull in diagnosing SAA in fetuses from about 95 days of gestation. Except for palatoschisis, the findings correspond to those on SAP in Charolais cattle (9). The fetal findings fit the hypothesis of an inherited neuromuscular dysfunction early in gestation as primary cause. Kyphosis, arthrogryposis, disturbed ossification and arachnomelia would be secondary effects. A similarity with the human Marfan syndrome as discussed in (7) seems rather improbable.
The observed ratio of SAA fetuses (8 out of 31) supports strongly the hypothesis of a single autosomal recessive gene with a very high degree of penetrance. This proportion indicates also that SAA embryos and fetuses are not exposed to a higher mortality than normal, at least between conception and transfer and during the first three to four months of gestation. It should be reminded, however, that all selected carriers had earlier had typical SAA progeny. This selection could also explain why the observed ratio fits the theoretical one so well. Field data, however, confirm the high penetrance of this defect (10) in contrast to SAP (5).

The results of the trial meet all expectations and the routine test for suspected A.I. bulls can be run exactly in the same way. The number of fetuses to be examined should be at least 16 in order to get 1% or less error probability under the assumption of complete penetrance. The fetuses should be collected at 95 to 100 days of pregnancy. If enough time is left for testing a bull, it is appropriate to proceed in successive steps of 4 to 5 pregnancies; as soon as the first SAA fetus occurs, the bull is recognized as a carrier and additional observations do not bring any additional information.

The routine test has already been started for two A.I. bulls. This combination of Mendelian genetics, embryo transfer and fetus examination is a useful tool in an A.I. breeding program. With this test it will be possible to significantly reduce the occurrence of a severe recessive defect without too large a loss in genetic improvement of economic traits.

REFERENCES

1 Brem, G., 1985: Personal communication.


Table 1: Number of offspring required to reach a given error probability (≡ probability of not detecting a heterozygotic carrier).
Assumed recessive frequency: 0.02

<table>
<thead>
<tr>
<th>Error probability:</th>
<th>Number of offspring from random sampled cows</th>
<th>Daughters of known carriers</th>
<th>Known carrier cows</th>
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<tbody>
<tr>
<td>P ≤ 0.05</td>
<td>304</td>
<td>23</td>
<td>11</td>
</tr>
<tr>
<td>P ≤ 0.01</td>
<td>467</td>
<td>34</td>
<td>16</td>
</tr>
<tr>
<td>P ≤ 0.001</td>
<td>697</td>
<td>51</td>
<td>24</td>
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Table 2: Age and number of fetuses examined for SAA

<table>
<thead>
<tr>
<th>Age (days)</th>
<th>Total</th>
<th>Number of fetuses positive</th>
<th>Uncertain</th>
</tr>
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<tbody>
<tr>
<td>124</td>
<td>3</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>116</td>
<td>2</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>110</td>
<td>4</td>
<td>1</td>
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<tr>
<td>105</td>
<td>5</td>
<td>1</td>
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</tr>
<tr>
<td>98</td>
<td>5</td>
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</tr>
<tr>
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<tr>
<td>87</td>
<td>8</td>
<td>1</td>
<td>3*</td>
</tr>
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</table>

Total 31 6 3*

after radiography* 31 8 -

(male: female) (11 : 20) (5 : 3) (- : -)

* with x-ray diagnosis, 2 out of the uncertain cases turned out to be positive, 1 negative.
Fig. 1: Stillborn SAA calf

Fig. 2: 98 day old SAA fetus

Fig. 3: 116 day old normal fetus (radiograph)

Fig. 4: 116 day old SAA fetus (radiograph)