Effects of Complex Vertebral Malformation on Fertility in Swedish Holstein Cattle

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Introduction

Complex Vertebral Malformation (CVM) is an autosomal recessive inherited defect in the Holstein breed first described in Denmark in 1999 by Agerholm et al. (2001). From September 2000 a marker test for the CVM-defect has been available and from February 2001 a direct gene test (Bendixen et al. 2002). All breeding bulls in Sweden have been tested since the tests became available and carriers are no longer used.

Various anomalies may be present in CVM affected calves. A diagnosis of CVM must, however, be based on the presence of malformation of the vertebral column, low weight for gestational age and arthrogryposis (Agerholm et al. 2001). Additional malformations i.e. cardiac defects are present in many cases. Nielsen et al. (2002) reported that 77% of CVM affected foetuses were aborted prior to gestation day 260. Thus the largest economical impact is not related to late term delivered calves but losses of pregnancies due to the large number of aborted foetuses. This leads to economic losses due to prolonged calving intervals and/or a too early and involuntary culling of cows.

The aim of this study was to investigate if Swedish Holstein bulls heterozygous for the CVM gene have reduced reproductive performance measured as non-return rate (NRR) and as a daughter fertility index.

CVM; congenital defect; non-return rate; dairy cattle.
Materials and methods
The NRR of Swedish Holstein bulls, used in breeding by Svensk Avel and Skånesemin, were compared according to the CVM genotype of the sire. Sires born between 1995 and 1999 were included if a minimum of 75 inseminations had been performed and the CVM genotype had been established. Out of the 375 bulls a total of 228 matched these criteria, of which 53 were CVM-carriers and 175 had a normal genotype or assumed to be negative since no bull in their descent (for at least three generations) was a known carrier. The CVM genotype of cows was not known but all inseminations were randomly distributed over cows with a restriction of an inbreeding level not exceeding 3% in the calves born. Relative breeding values (RBV’s) for NRR was obtained from the Swedish Dairy Association. These are calculated setting a mean of 100 and a standardised deviation of 7 units.

The SAS-program (SAS Institute Inc. 2000) was used to determine the effects of the paternal CVM genotype, the paternal grandsire, and the birth year of bull on 28, 56 and 168 days NRR. In addition, effect of paternal CVM genotype on daughter fertility was investigated. The daughter fertility is expressed in an index of several fertility measures (interval from calving to first insemination, number of inseminations per serviced cow, heat intensity and

Table 1. Frequency of CVM-carriers grouped according to birth year for 228 Swedish Holstein bulls

| Birth year of bull | CVM-carriers N | Frequency % |
|--------------------|----------------|-------------|
| 1995               | 0              | 0           |
| 1996               | 3              | 20.0        |
| 1997               | 18             | 21.7        |
| 1998               | 23             | 30.3        |
| 1999               | 9              | 18.0        |

Figure 1. Relative breeding values (RBV’s) for 28, 56 and 168 days non-return to service rate (NRR) in 175 homozygous normal bulls and in 53 CVM-carrier bulls (Mean ± SD).
treatments for reproductive disturbances) from the heifer period, first and second lactation.

Results
Altogether 228 bulls were analysed of which 53 bulls, i.e. 23%, were confirmed CVM-carriers. There was an increase in the number CVM-carriers during the studied period until birth year 1999 (Table 1).

In Fig. 1 RBV’s for 28, 56 and 168 days NRR are depicted for CVM-carrier bulls and non-carrier bulls. The standard deviation of the mean increased with increasing days NRR and especially so in the carrier group.

A statistically significant difference between CVM-carriers and non-carriers in the RBV for NRR was obtained for 168 days NRR (101.1 ± 0.9 vs. 103.1 ± 0.6, p<0.05). There was no difference for 28 days NRR whereas the difference approached significance for 56 days NRR (Table 2). The paternal sire significantly influenced the measures of NRR but the effect of birth year of the bull was not statistically significant.

No statistically significant effect of paternal CVM genotype on the daughter fertility index was shown.

Discussion
A large variation in NRR was observed for both carriers and non-carriers of CVM and consequently it was not possible to determine the CVM genotype based on individual NRR values.

NRR is often used as an early obtainable measure of the fertility of a bull. It is a crude measure of fertility though, since it is only based on the proportions of animals being re-inseminated. The measure is overestimated compared with the true proportion of animals becoming pregnant. No difference was seen between carriers and non-carriers of CVM on NRR values up to 28 days, indicating minimal early embryonic losses due to CVM. A tendency for increased return to service was recorded within 56 days for CVM-carriers and at 168 days a significant difference was found. This finding is consistent with the findings by Nielsen et al. (2002) in Danish Holstein and with recent studies of German Holsteins (Konersmann et al. 2003), and of French Holsteins (Malher et al. 2003). In the latter study, return to service in various intervals after calving was studied in a large material from Brittany between the years 1998 and 2001. As in our study, the risk of return to service was significantly higher, in particular for late return. Possible reasons why there is no difference between the groups until gestation day 168 might be that there have to be a certain amount of pregnancy losses until the difference can be noticed. The cows also have to return to service after resorption or abortion of the conceptus and this has to be detected by the herdsmen.

A possible explanation to why there was no difference between the 2 groups in daughter fertility is that the Swedish daughter fertility index is a complex measure composed of several different sub-traits of fertility, of which the only trait that could be affected by CVM is the number of inseminations per serviced cow.

The number of CVM carriers depends on the usage of semen from carrier bulls and increases until preventive measures are taken. Programs for eradication of the CVM defect have been

| NRR days | Homozygous normal (LS-mean ± SE) | CVM-carriers (LS-mean ± SE) | P-value |
|----------|---------------------------------|-----------------------------|---------|
| 28       | 100.7 ± 0.3                     | 100.2 ± 0.4                 | 0.277   |
| 56       | 101.8 ± 0.4                     | 100.5 ± 0.6                 | 0.076   |
| 168      | 103.1 ± 0.6                     | 101.1 ± 0.9                 | 0.039   |
adopted in most countries and in Sweden an eradication program has been run since September 2000. An increase in the number of heterozygotes for the CVM-gene until then was therefore expected and the frequency tended to increase until birth year 1999 of bulls. In a German study (Konersmann et al. 2003), an increase in the CVM frequency from 1997-2000 was shown with a drop in 1998. The frequency depends largely on the date of birth and the usage of bull fathers. Their analysis comprised 957 sires and the average frequency over the period of CVM affected animals was 13.2%.

There are few reports in the literature of CVM calves born after full-term gestation. Clinical cases of CVM calves have been found in Denmark (Agerholm et al. 2001), the United States (Duncan et al. 2001), the United Kingdom (Revell 2001) and Japan (Nagahata et al. 2002), but cases of CVM has not been reported from Germany (Konersmann et al. 2003). In a post-mortem investigation of stillborn calves from commercial herds performed in 2000/2001 in Sweden (unpublished), a 261 days old foetus with lesions consistent with CVM was found.

If the losses in Swedish Holsteins should be calculated a rough estimate could be based on a frequency of CVM carriers of 23% in both bulls and cows. Provided an even usage of CVM carriers over cows this means 5.3% carrier inseminations of which 25%, i.e. 1.3% of all inseminations, result in foetuses homozygous for the gene. In the Swedish Holstein population (170,000 milk recorded cows), this corresponds to a loss of 2200 calves/year due to a reduced viability of foetuses. Possibly the frequency of carriers was somewhat lower in cows than in bulls, which would overestimate the losses. In addition to lost foetuses, a number of cows might have been involuntarily culled due to prolonged calving intervals.

All Holstein-bulls are nowadays tested for the CVM defect and carrier bulls are no longer used in Sweden, as in many other countries, and consequently the number of heterozygotes for this defect will rapidly decrease. In the middle of the 1990's the Bovine leukocyte adhesion deficiency (BLAD) defect in Holsteins was dealt with in the same way. New defects will certainly turn up in the future. Therefore, it is important to report all kinds of malformations and to have national control programs for congenital defects in order to avoid multiplication of deleterious genes.

**In conclusion** this study showed that carriers of the CVM-gene had an inferior NRR compared with non-carrier bulls reflecting an increased rate of intra-uterine mortality. The risk of return to service was significantly higher in particular for late returns.

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Malher X, Beaudreau F, Philipot JM: Effects of sire and dam genotype for complex vertebral malfor-
Complex vertebral malformation (CVM) är en genetisk defekt i Holsteinrasen som först rapporterades från Danmark 1999. Den orsakar fosterdödlighet genom hela dräktighetsperioden och leder på så sätt till ekonomiska förluster genom omlöpningar och ofrivillig utslagning av kor. Ett markör-test för defekten har funnits tillgängligt från september 2000 och en direkt gen-test sedan februari 2001. Alltsedan dess testas alla tjurar i Sverige för defekten och bärare används ej i aveln. Syftet med detta studie var att retrospektivt undersöka om man kunde se en skillnad i fruktsamhet mellan Holstein tjurar som var bärare av CVM-genen jämfört med icke-bärare. Alla CVM-testade tjurar som använts i den svenska AI-organisationen som var födda mellan 1995 och 1999 ingick i studien. Analysen omfattade 228 tjurar varav 53 stycken d.v.s. 23% var bärare av CVM-genen. En statistisk signifikant skillnad i 168 dagars icke-omlöparfrekvens erhölls mellan bärare och icke-bärare (101,1 ± 0,9 vs. 103,1 ± 0,6, p<0,05). Det var ingen skillnad mellan grupperna för 28 dagars NR men den nårade sig signifikans vid 56 dagars NR. Ingen effekt av CVM på dotterfruktsamsindexet kunde påvisas. Det beror sannolikt på att detta index är sammansatt av flera olika egenskaper varav CVM bara kan påverka antalet inseminationer per insemineringsomgång. Sammanfattningsvis visade studien att bärare av CVM-genen hade en signifikant sämre fruktsamhet mätt som icke-omlöparfrekvens, jämfört med CVM-fria tjurar. Eftersom nya defekter uppstår vartefter illustrerar detta exempel vikten av att rapportera in alla misstänkta missbildningar och av att ha kontrollprogram för genetiska defekter så att åtgärder kan sättas in på ett tidigt stadium innan de förorsakat alltför stora negativa konsekvenser.

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