Genetic aspects of twinning in cattle

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Abstract. Twinning in cattle ranges from about 1% for beef breeds to about 4% for dairy breeds. The incidence of double births may have both positive and negative effects, which mainly depends on the purpose for which the cattle are raised. Because of freemartinism, as well as management problems connected e.g. with a greater risk of dystocia and retained placenta, it is an undesirable trait in dairy herds. In beef cattle, however, twinning can considerably increase the efficiency of production. Low heritability, a long generation interval for progeny testing, sex-limited expression and an unfavourable correlation with milk yield make twinning difficult to control by selection. Hence, it is the type of trait for which the identification of the genetic marker - quantitative trait loci (QTL) linkage and the implementation of marker-assisted selection in breeding strategies are expected to be especially beneficial. Searching for QTL influencing the reproductive rate in cattle was performed mainly in the US Meat Animal Research Center twinning herd and in the commercial Norwegian cattle population. Among several genome regions that appear to control twinning and ovulation rates, the most interesting seem to be chromosomes 5, 7, 19 and 23.

Key words: cattle, ovulation rate, quantitative trait loci, twinning rate.

Introduction

Cattle (Bos taurus) are a uniparous species meaning that, in most cases, females produce only one offspring per pregnancy. Twinning occurs relatively rarely, with the frequency generally not exceeding 1% in most of beef herds, in which selection on this trait has not been practised (for review, see RUTLEDGE 1975). In dairy herds, the incidence of double births is higher (on average 4-5%), and is strongly
affected by age and parity of the dam, ranging from about 1% for heifers to nearly 10% for older cows. Twinning rate can increase over a 10-year age of cows period, and the largest increase was observed between the first and second parity (BERRY et al. 1994, CADY, VAN VLECK 1978, KINSEL et al. 1998, NIELEN at al. 1989, RYAN, BOLAND 1991). Twinning rate is also slightly influenced by seasonal effects, with a trend toward more multiple births during the spring (CADY, VAN VLECK 1978, KARLSEN et al. 2000) or autumn months (GREGORY et al. 1990a). The nature of seasonal effects, although uncertain, is thought to be connected with changes in temperature, the duration of daylight or in feeding at conception.

Freemartinism in cattle

Twinning rate in cattle is closely related to the ovulation rate. The monozygotic, genetically identical twins, due to the spontaneous single embryo division, were estimated to comprise less than 10% of all double births (CADY, VAN VLECK 1978, ERB, MORRISON 1959, JOHANSSON et al. 1974). A majority of bovine twins are of the dizygous type, resulting from the ovulation and fertilization of two oocytes. Dizygotic twins are no more alike than siblings with the same parents born during different gestations, and can be of the same or opposite sex. In the latter situation, the freemartin effect often occurs.

"Freemartin" is the term used to describe the infertile females born as a co-twin to the males. Masculinization of the internal reproductive tract of these animals results from the exposure of the female foetus to the blood of the male twin in the uterus.

In cattle, shortly after the implantation of twin pregnancies, placental vascular anastomoses of the two foetuses usually occur. This allows for the exchange of blood constituents between twins. The placentas are fused before the sexual differentiation of foetuses. Also, the differentiation of gonads starts several days earlier in males than in females. Thus, when the twin foetuses are of different sex, sex determining factors from the developing male gonad are transported to the female foetus through the common blood vessels and may suppress the development of the reproductive organs of the heifer. In most cases, freemartin gonads develop into ovotestes that contain both ovarian and testicular tissues. The remainder of the internal reproductive tract is usually underdeveloped and can vary from an almost normal female to that very similar to a male.

Abnormalities of the female twin are most likely caused by the anti-Mullerian hormone (AMH) (VIGIER et al. 1988, 1991). AMH, secreted by foetal Sertoli cells, is responsible for the regression of the Mullerian structures in males as a part of their normal sexual development. The occurrence of seminiferous tubules in the prospective foetal ovaries, exposed in vitro to purified AMH suggests that this hormone can also be involved in the morphological differentiation of testes.
In bovine heterosexual litters, the regression of Müllerian ducts occurs simultaneously in males and freemartins between 50 and 80 days of pregnancy. The masculinization of the gonads and the development of the Wolffian ducts occur later, around day 90. Both twins exhibit high serum AMH concentrations, whereas the gonadal production of this hormone in females is very low (VIGIER et al. 1984).

In addition to the transfer of hormones, anastomoses of placental vessels allow for the interchange of haemopoetic stem cells between foetuses. In heterosexual twins it results in the formation of sex chromosome chimerism (60, XX/XY) in peripheral blood leucocytes (BASRUR et al. 1970). Therefore, a cytogenetic study can be a useful approach for the detection of freemartinism (BHATIA, SHANKER 1985). The other diagnosing methods include blood typing (JUSTI et al. 1995) and the PCR (JUSTI et al. 1995, SHELLANDER et al. 1992). In freemartins, the ratio of XX to XY karyotypes in cultures of blood lymphocytes varies considerably from individual to individual, and it is not related to the degree of masculinization (GREENE et al. 1977). The bulls co-twin to heifers are also chimerical, but their genital organs are normal. However, on account of the poor quality of their semen, the fertility of some XX/XY males can be decreased (DUNN et al. 1979, STAFFORD 1972, GEWİTOŃSKI et al. 1991). Cytogenetic investigations of bulls used in artificial insemination showed that leucocytal chimerism, with frequency ranging from 1% to 4.2%, is more common than other chromosomal aberrations (KOVÁCS 1996, SEGUIN et al. 2000, GEWİTOŃSKI et al. 1991). Because the reproductive performance of chimeric males is often not satisfactory, it is postulated to exclude them from AI Stations.

Freemartinism occurs more frequently in cattle than in any other species. Between 82.5% (ZHANG et al. 1994) and 92% (BUOEN et al. 1992) of heifers from mixed sex twin pregnancies are freemartins. The remaining 8-17.5% of females develop correctly, presumably because the placental vessels fail to fuse or because the fusion occurs after the critical period of reproductive organ differentiation. The background of the placental anastomosis development is not clear. There are indications that breed type may influence the incidence of chimerism in cattle (SUMMERS et al. 1984). In sheep, the chimerism frequency in individuals originating from heterosexual twins ranges from 4.8% (MATEIKA et al. 1987) to 25.14% (KESZKA et al. 2001). The distinct differences observed between breeds indicate the hereditary tendency of the placental anastomosis development. Moreover, pedigree analyses suggest that the formation of anastomoses in sheep is controlled by a single dominant gene (KESZKA et al. 2001, SZATKOWSKA, GEWİTOŃSKI 1996).

**Management of twinning cows**

Multiple births bring with them a greater risk of dystocia, and consequently a decreased survival rate of calves at birth (ECHTERNKAMP, GREGORY 1999,
A high level of difficult calvings results from an abnormal presentation of one or both foetuses at parturition rather than from their large physical size, because twins are lighter at birth in comparison to singles (GREGORY et al. 1990b, GREGORY et al. 1996, GUERRA-MARTINEZ et al. 1990). Cows delivering twins also have a higher risk of premature calving and retained placenta (ECHTERNKAMP, GREGORY 1999, EDDY et al. 1991, GREGORY et al. 1990b, GUERRA-MARTINEZ et al. 1990, NIELEN et al. 1989), and of a reduction in future reproductive efficiency (CHAPIN, VAN VLECK 1980, EDDY et al. 1991, GREGORY et al. 1990b). Some of these problems can be overcome with intensive management of twin-producing dams and their calves during the calving season. The required management alterations include pregnancy checks for the identification of cows gestating twins and obstetrical assistance to facilitate the delivery of twin calves and to increase their neonatal survival.

**Effect of twinning on beef cattle productivity**

Double births provide an opportunity to improve the efficiency of beef production for producers who have the skills to manage the complications associated with twinning. A low reproductive rate is one of the main factors limiting productivity in beef cattle. Over 50% of total production costs are attributed to the maintenance of reproducing females (a comparable value in meat chickens is about 3%) (DICKERSON 1978). Twinning could spread these costs on approximately 60-70% more calf weight at weaning produced per one calving (GREGORY et al. 1996, GUERRA-MARTINEZ et al. 1990, DE ROSE, WILTON 1991). GUERRA-MARTINEZ et al. (1990) estimated that even taking into consideration higher labour and veterinary costs, the increase in the efficiency of producing beef by twinning can reach 24%. Furthermore, obtaining infertile freemartins is of no importance in beef cattle, because most of calves are destined for slaughter. Due to higher carcass quality grades in comparison with normal females (GREGORY et al. 1996), producing freemartins may even be favourable.

**Selection for twinning rate**

The double birth incidence is difficult to control by selection because of the long generation interval for progeny testing, sex-limited expression and low heritability (h²). In dairy cattle, selection against twinning, as an undesirable trait, interferes with the unfavourable correlation between twin birth frequency and milk production. High milk yield as a risk factor for twinning was indicated in several reports (BAR-ANAN, BOWMAN 1974, KINSEL et al. 1998, MAIJALA, OSVA 1990, MAIJALA, SYVÄJÄRVI 1977). FRICKE, WILTBANK (1999) and WILTBANK et al. (2000) described a direct relationship between high milk production and the increased incidence of double ovulation, which may subsequently result in increased twinning. Although the mechanism that increases double ovulations
in high producing cows remains unknown, WILT BANK et al. (2000) proposed an explanation of the observed association. Their model assumes that an increased steroid metabolism could be responsible for multiple ovulations. In high efficiency lactating cows, high feed intake increases blood flow to the digestive tract and to the liver. Because blood entering the liver, the primary site of steroid metabolism, is cleared of the steroids, increased blood flow can influence the number of dominant follicles by affecting estradiol and, indirectly, FSH concentrations.

Twinning rate is generally considered as a trait with low heritability. The value of $h^2$ was estimated to range from less than 0.01 to 0.09 (CADY, VAN VLECK 1978, GREGORY et al. 1997, KAR LSE N et al. 2000, MAIJALA, OSVA 1990, RON et al. 1990, SYRSTAD 1984, VAN VLECK, GREGORY 1996). Ovulation rate, the trait closely related to twinning, when considered as a single observation, also has a low heritability of 0.07-0.11 (ECHTERNKAMP et al. 1990a, VAN VLECK, GREGORY 1996, GREGORY et al. 1997). However, when multiple oestrous cycles are taken into consideration, $h^2$ is considerably higher. For eight oestrous cycles, it rises to 0.34-0.38 (ECHTERNKAMP et al. 1990a, GREGORY et al. 1997). The genetic correlation between twinning and ovulation rates ranges from 0.75 to almost 1.0 (GREGORY et al. 1997, VAN VLECK et al. 1991). It is also important that measurements of ovulation rate in puberal heifers are available a year earlier than a single measurement of twinning rate at first parturition, when twinning frequency is lower than at older ages.

In the late 1970s and early 1980s some experiments, e.g. in New Zealand (MORRIS, DAY 1990b) and at the United States Department of Agriculture – Meat Animal Research Center (USDA-MARC) (GREGORY et al. 1990b) were implemented to study twinning in cattle. The main goal of these experiments was to determine the potential for genetic improvement in reproductive rate. The use of ovulation rate as an indirect selection criterion led to a significant increase in the double birth frequency, especially in the multi-bred composed USDA-MARC population. Ovulation rate was determined in puberal heifers by rectal palpation of the corpora lutea, starting at an average age of 11 months and continued for eight oestrous cycles (ECHTERNKAMP et al. 1990a). The frequency of multiple births has been increasing at the rate of approximately 2% per year in this herd. Originally, in 1982, the average twinning rate was 3.4% (VAN VLECK, GREGORY 1996). At the latest report, it exceeds 35% (ECHTERNKAMP, GREGORY 1999). Thus, after nearly 20 years of selection, the frequency of twin births has not yet reached the level (of at least 40%) that is required for the consideration of a twinning technology by the beef cattle industry (GREGORY et al. 1997).

**Genetic background of the reproductive rate in cattle**

Numerous reports speculated about the existence of the single gene with a large effect on the reproductive rate in cattle (BAR-ANAN, BOWMAN 1974, MORRIS, DAY 1986). Often these speculations followed from the identification of animals
with extremely high or low percentages of double births among their daughters, compared with the mean for the population. SYRSTAD (1984) and GREGORY et al. (1990a), however, while examining the daughter twinning rates for the sons of sires with an exceptional twin births frequency, found no evidence to support the hypothesis of the major gene existence. The absence of bimodality in progeny twinning rates, which would be expected if a gene with a large effect was segregating, has been recognized as an indication that this trait is not under the control of a single locus. Also in other studies (MORRIS, DAY 1990a, MORRIS, FOULLEY 1991, RON et al. 1990), no evidence was found for the hypothesis that litter size is affected by a major gene. Thus, twinning rate in cattle is likely to be inherited as a typical quantitative trait, controlled by the combined action of many genes modified by environmental factors.

Recently, the development of highly polymorphic markers and medium density linkage maps (BARENDSE et al. 1997, KAPPES et al. 1997) has opened the possibility to identify genes underlying genetic variation for complex quantitative traits (quantitative trait loci – QTL) in cattle. Once mapped, QTL can be manipulated by marker assisted selection (MAS), in which closely linked genetic markers, co-inherited with the gene affecting a quantitative trait, are used to select for the desired allele of that gene. Ultimately, it may be possible to actually isolate QTL which have been mapped by the application of the "reverse genetics" procedures.

Figure 1. Approximate location of prospective QTL for the twinning rate (tw. r.) and ovulation rate (ov. r.) on the bovine chromosomes. Arrows and vertical lines indicate results obtained by single marker regression and multimarker regression, respectively. Based on the following studies:

A BLATTMAN et al. (1996) – 3 families from the USDA-MARC twinning population; single marker regression, within family analysis, 77 markers. B KIRKPATRICK et al. (2000) – 1 family from the MARC twinning population; single markers regression, 14 markers; for chromosomes with significant results locus scan – multimarker regression with the use of additional markers. C KAPPES et al. (2000) – 10 families from MARC twinning population; multimarker regression, within family analysis; genome scan (g) with the use of 273 markers; for chromosomes with significant results additional locus scan (l). D LIEN et al. (2000) – 6 families from the commercial Norwegian cattle population; multiple marker regression, both across and within family analyses, 293 markers.
For cattle, the focus was initially on the identification of genes involved in milk production. Although some markers associated with other traits were also found, the number of these studies is limited. Most QTL reports on bovine ovulation rate are mainly from the USDA-MARC twinning herd (Blattman et al. 1996, Kappes et al. 2000, Kirkpatrick et al. 2000). Moreover, searching for genes affecting twinning rate was performed in the commercial Norwegian cattle population (Lien et al. 2000). Several different regions of chromosomes appear to influence reproduction rate (Figure 1). The most interesting seem to be chromosomes 5, 7, 19 and 23.

In the USDA-MARC population, different methods were used to detect genes affecting ovulation rate in cattle. Initially, a single-marker analysis with the use of 77 markers (43-45 informative markers per family) was performed in three large grandsire families (Blattman et al. 1996). Kirkpatrick et al. (2000), using 14 other markers, continued the examination of one of these families. For significant results, the locus scan by multimarker regression was carried out. This approach led to the identification of some regions connected with the ovulation rate on chromosomes 5, 7, 10, 19 and 23. Kappes et al. (2000) applied the multimarker regression for the whole genome scan. The analysis (granddaughter design), performed within 10 families with the use of 273 genetic markers, revealed 41 significant results. Among 10 chromosomal regions with the highest significance value, the additional locus scan (daughter design) confirmed only one, located on chromosome 5, at the relative position 40 cM. The second significant peak (on BTA25) was interpreted as not supporting the results of the genomic scan, because effects of two alleles in the daughters of the sire were reversed relative to sons of the sire.

Multimarker regression (293 markers, mostly from the Norwegian cattle map (Våge et al. 2000)) was used for the autosomal genome scan in the Norwegian research (Lien et al. 2000). Potential QTL for the twinning rate were detected on chromosomes 5, 7, 12 and 23. The QTL with the highest significance was observed close to the IGF1 locus in the central part of chromosome 5.

After the identification of the QTL for reproductive rate, the next step should be the selection of candidate genes for this trait. Genes that influence ovulation rate are likely to be involved in follicular recruitment and development or ovulation. Those affecting twinning rate may also be involved in fertilization, implantation and embryo survival.

Two genes with large effects on the ovulation rate, the Booroola fecundity gene (FecB) and the Inverdale fecundity gene (FecX), have recently been identified in sheep (Galloway et al. 2000, Mulsant et al. 2001, Souza et al. 2001, Wilson et al. 2001). It was shown that a mutation in the highly conserved domain of the bone morphogenetic protein receptor IB (BMPR-IB) gene located on ovine chromosome 6 is fully associated with the hyperprolific phenotype of Booroola Merino ewes. The amino acid substitution at position 249 of the BMPR-IB protein impairs its inhibitory effect on granulose cell steroidogenesis leading to an ad-
advanced maturation of follicles (MULSANT et al. 2001, SOUZA et al. 2001, WILSON et al. 2001). The causative mutation for Inverdale fecundity has been identified within the bone morphogenetic protein 15 (BMP15) gene on the X chromosome. In heterozygous animals, the activation of only one copy of BMP15 allows for more than one follicle to be selected for ovulation by reducing the number of mitotic divisions and the amount of steroid or inhibin produced by each follicle, and thereby delaying suppressive effects on plasma FSH concentrations. This then results in a larger proportion of antral follicles with granulosa cells that become responsive to LH, and thus in an increase in the ovulation rate. Ewes that are homozygous for inactivating mutation, however, are infertile due to a block in follicular growth (GALLOWAY et al. 2000). Sheep chromosomes 6 and X correspond to bovine chromosomes 6 and X, respectively (ECHARD et al. 1994). As yet, however, there are no indications that chromosomes 6 and X could be connected with twinning or ovulation rates in cattle. Also, no obvious candidate genes for reproductive rate have yet been designated in the genome regions bearing putative QTL. However, the growth hormone (GH) gene, located on chromosome 19, could be taken into consideration.

As reviewed by HULL, H ARVEY (2001), the growth hormone influences fertility in many different ways. GH modulates gonadotropin-independent early folliculogenesis and gonadotropin-dependent late folliculogenesis by increasing cell proliferation and inhibiting atresia. Moreover, it enhances oocyte quality by accelerating and coordinating cytoplasmic and nuclear maturation, affects the production of ovarian steroids, and plays a facilitatory role in ovulation. Some effects of the growth hormone are mediated by the insulin-like growth factor I (IGF1), which is synthesized in the liver and ovary in response to GH. The IGF1 gene, located on chromosome 5 in the region where QTL controlling twinning rate was found (LIEN et al. 2000, Figure 1), could be considered as another candidate gene for this trait. IGF1 was shown to stimulate the granulosa cell mitogenesis and the steroidogenesis of cultured ovarian cells (for review, see Spicer, ECHTERNKAMP 1995). The study of ECHTERNKAMP et al. (1990b) provided evidence suggesting that double births in cattle are associated with an increased concentration of IGF1 both in the serum and in the follicular fluid. These data indicate that IGF1 plays a role in the regulation of folliculogenesis and may be involved in multiple ovulations in cattle. To date, however, no associations between polymorphisms in coding or regulatory regions of IGF1, as well as GH genes, have been found.

Conclusions

Twinning technology, undesirable in dairy cattle, has the potential to improve the efficiency of beef production. However, intensive management for twin producing cows is necessary to overcome problems associated with double births, in-
cluding a higher risk of dystocia and lower calf survival. The increase in twinning rate, despite low heritability of this trait, can be achieved through selection, when multiple observations of ovulation rate are used as the indirect selection criterion. Also, the identification of the QTL for reproductive rate makes it possible to improve the response to selection by selecting genetically superior animals on the basis of the DNA markers.

REFERENCES


