CONCLUSIONS

This thesis has investigated familial dizygotic (DZ) twinning. Its primary aims were to measure empirical recurrence risks for DZ twinning, to find out whether DZ twinning is inherited and, if so, to identify a genetic model underlying the familial occurrence of DZ twin pairs and to study the genetic relationship between artificial induction of ovulation and DZ twinning.

DZ twinning is the consequence of multiple ovulation in women (Milham, 1964). The underlying biological mechanism causing multiple ovulation is so far unknown. However, it has been suggested that in DZ twin mothers increased secretion of pituitary gonadotrophins, such as follicle stimulating hormone (FSH), is responsible for ovarian hyperstimulation. This increase of plasma gonadotrophins in DZ twin mothers is in itself considered as the result of either a higher sensitivity of the pituitary gland for hypothalamic gonadotrophins releasing hormone (GnRH) or of an increased hypothalamic secretion of the GnRH.

Familial twinning was studied in pedigrees of DZ and monozygotic (MZ) including both spontaneous and induced proband twin pairs, ascertained through two population-based twin registers, the Netherlands Twin Register (NTR) and the East Flanders Prospective Twin Survey (EFPTS). Relative risks for the birth of a DZ twin pair were significantly increased along the maternal line of a DZ proband pair. More specifically, parents and maternal grandparents of the mother of a spontaneous DZ twin pair in the Netherlands had 1.5 times more chance of becoming parents of a DZ twin pair than random parents in the Netherlands. The parents and sisters of a mother of a spontaneous DZ twin pair had in East Flanders, Belgium, almost 2 times more chance of getting DZ twins. These
increased risks along the maternal line of a DZ twin pair, either in the Netherlands or Belgium, confirmed the results of earlier studies (Weinberg, 1902; Weinberg, 1909; Bonnevie and Sverdrup, 1926; Waterhouse, 1950; Wyshak and White, 1965; Nylander, 1970). The absence of any increased risks along the paternal line strongly opposed any contribution of the father of the DZ proband pair, which was suggested by Greulich (1934) and Parisi et al. (1983).

The relative risk for the birth of a MZ twin pair showed no clear or genetically interpretable pattern in the pedigrees of MZ twins. The familial clustering of MZ twin pairs was too weak to have a major effect on an epidemiological level. Although this observation did not contradict any genetic cause for MZ twinning, it was compatible with the viewpoint of Sedgwick-Harvey et al. (1977) and Olson-Segreti et al. (1978), who described a low penetrant autosomal dominant gene, causing MZ twins only in a limited number of families of MZ twins. On an epidemiological level, there was also no evidence to support any common origin for DZ and MZ twinning (Parisi et al., 1983; Gedda and Bencic, 1983; Derom et al., 1987) as DZ twinning risks were not increased in MZ twin pedigrees nor vice versa.

The segregation analysis of the familial clustering of spontaneous DZ twins modelled DZ twinning as "having DZ twins" and defined it as a trait with a female-specific expression. An autosomal monogenic dominant model described the data better than any other model under these assumptions. The dominant model contradicted the recessive hypothesis, suggested by several other studies on the genetics of twinning (Bonnevie and Sverdrup, 1926; Wyshak and White, 1965; Bulmer, 1970). The gene frequency was relatively high, 0.0352. It was estimated that approximately one in fifteen individuals was a gene carrier. However, only 10% of those female carriers will have a spontaneous DZ twin pair among their offspring by the end of their reproductive life. A possible explanation for the low penetrance of the trait is spontaneous abortions and early fetal death. Boklage (1990) demonstrated that approximately 75% of all singleton conceptions are aborted within the first six weeks of gestation. Boklage estimated that although multiple pregnancies represent about 12% of all natural conceptions, only 2% of all multiple conceptions will survive to term as twins and about 12% end with the birth of a singleton. Leridon (1977) estimates that approximately 42% of all conceptions survived to a clinical identified pregnancy. If both fertilized eggs had the same chance of surviving, this would mean that approximately 17.6%, the square of 42%, of all multiple conceptions would be recognized as a multiple pregnancy. This was only 7% above the estimated penetrance for gene carriers in the dominant model.

The segregation analysis of spontaneous DZ twinning in the maternal families of induced DZ proband pairs used the same phenotype as for the analyses on pedigrees of spontaneous DZ proband pairs. An autosomal monogenic dominant model was again favoured as the best explanation of familial DZ twinning. In fact, there was no heterogeneity in modelling between the maternal families of spontaneous and induced DZ proband pairs. This meant that the genetic model found in families of spontaneous DZ proband pairs also applies to families of induced DZ proband twins. This suggested that women bearing DZ twins after artificial induction of ovulation already have a genetic predisposition to DZ twinning.

By introducing parity into the modelling of DZ twinning, the phenotype was no longer defined as a binary trait. Indeed, parity dependent modelling took into account the number of offspring of each mother and the number of DZ twin pairs among those children. In this way, mothers with large number of offspring became more informative.
as they had more chance of expressing the trait. However, our results of the parity dependent segregation analysis were disappointing. The difficulties encountered during the analyses clearly indicate that larger and more informative datasets are required to model parity in the segregation analysis of DZ twinning. The main lesson, drawn from these experiments, was that the sporadic or non-genetic model was clearly insufficient to explain the familial clustering of DZ twinning.

The genetic predisposition to multiple ovulation has been identified in two sheep models. A codominant model, known as the Booroola fecundity gene (FecB), has been described in strains of CSIRO Booroola Merino Sheep in Australia (Piper and Bindon, 1982). Genotypes of the individual ewes were identified as a function of the number of ripening follicles during the menstrual cycle. Homozygous and heterozygous carriers were defined as those ewes with ovulation rates of 5 or more and 3 or 4 follicles per cycle respectively (McNatty et al., 1986). Non-carriers had ovulation rates which were not higher than 2 follicles per menstrual cycle. This additive effect has also been observed in plasma concentrations of gonadotrophins. Indeed, homozygotic ewes had significantly higher FSH and LH plasma levels than heterozygous ewes, which in turn had significantly higher plasma levels than non-carriers (Robertson et al., 1984; McNatty et al., 1987; McNatty et al., 1989). In addition, the mean litter size of each ewe increased with 1.5 lambs with each copy of the affected allele. (Piper et al., 1985). The absence of any expression of the affected allele in males suggested that the expression of the gene is female-specific (Bindon and Piper, 1986). Montgomery et al. (1993) have localized this autosomal codominant gene (FecB) to a region homologous to the human chromosome 4 and more precisely region q21-q25.

An X-linked model, called the Inverdale gene (FecX1), has been observed in strains of Romney Sheep in Australia (Davis et al., 1991). The ovulation rate of heterozygous ewes was on average 1 follicle higher than non-carriers. However, ewes, homozygous for the Inverdale gene, were infertile due to bilateral ovarian hypoplasia (Davis et al., 1992). The autosomal fecundity gene in Booroola sheep (FecB) supports the autosomal model for DZ twinning, found in families of spontaneous DZ proband pairs. Our segregation favoured a dominant model, in contrast with the observed codominant model in Booroola Merino Sheep. This difference in models can be explained by the phenotype definition. In humans, DZ twinning is assumed as proof for multiple ovulation, while for Booroola sheep, the number of ripening follicles was measured through ultrasound. The rejection of an X-linked model for DZ twinning in pedigrees of DZ twin pairs contradicted the X-linked Inverdale gene (FecX1) observed among Romney Sheep. Our data did not support any X-linked inheritance for twinning. However, increased twinning frequencies have been observed in pedigrees of fragile-X patients (Fryns, 1986; Sherman et al., 1988) and was predominantly caused by DZ twinning (Thomis et al., 1993). Our results were supported by earlier literature on the inheritance of DZ twinning, by endocrinological observations and by animal models. Our proband twin pairs were representative for their respective populations in terms of zyosity and the collected pedigrees were representative of all families of twins, registered in either the NTR or the EFPTS. Given DZ twinning frequencies are relatively comparable among caucasoid populations, the autosomal monogenic dominant model for familial DZ twinning in the Netherlands and Belgium may well reflect the familial clustering for any caucasoid populations. The absence of heterogeneity in the genetic modelling of DZ twinning between Dutch and Belgian pedigrees and between spontaneous and induced DZ proband pairs gave additional support. However, one model in segregation analysis does
not automatically imply only one gene. Indeed, the observed dominant mode of inheritance can be caused by a single gene, or several rare dominant genes that have a summed gene frequency of 0.0352 and a mean penetrance of 0.1025. Mathematical modelling would not distinguish between these.

This work was innovative on four different levels. First, the collected dataset was no longer a mixture of volunteering families, isolates or families with many twin pairs. Secondly, zygosity was determined on each individual twin pair and was no longer assessed on a population scale using Weinberg's rule. Third, the genetic analysis introduced pedigree analysis as a new tool in the investigation of the inheritance of twinning. Fourth, this study was the first to investigate increased frequencies of multiple births after artificial induction of the ovulation from a genetic viewpoint.

Further research on the genetics of DZ twinning should focus on the localisation of the DZ twinning gene. The cosegregation of the DZ twinning phenotype, "having DZ twins", and a number of candidate genes offers the most interesting perspective. Candidate genes are defined as those genes which most likely seem to be involved in the biological process, which results in the observed phenotype. Multiple ovulation was assumed as the underlying biological mechanism causing pregnancies of dizygotic twins. Consequently, hormones and their receptors involved in the endocrinological cycle associated with ovulation in females, have a high priority in the linkage analysis. In addition, the Alpha-1-antitrypsin inhibitor (AAT) has been suggested to increase fertility and to associate with the occurrence of multiple pregnancies. Indeed, Clark and Martin (1982) observed that the frequency of the S allele was twice as high in mothers of DZ twin pairs than in control mothers. The frequency of S and Z alleles on the AAT locus was almost three times as high in mothers of DZ twin pairs compared to control mothers in the Netherlands (Boomsma et al., 1992b). However, the underlying biological mechanism through which AAT causes DZ twins, is so far unknown. Finally the most important region to be studied for linkage analysis, involves 4q21-25, homologous to the region, in which the Booroola Fecundity gene (FecB) was located in Booroola Merino Sheep.