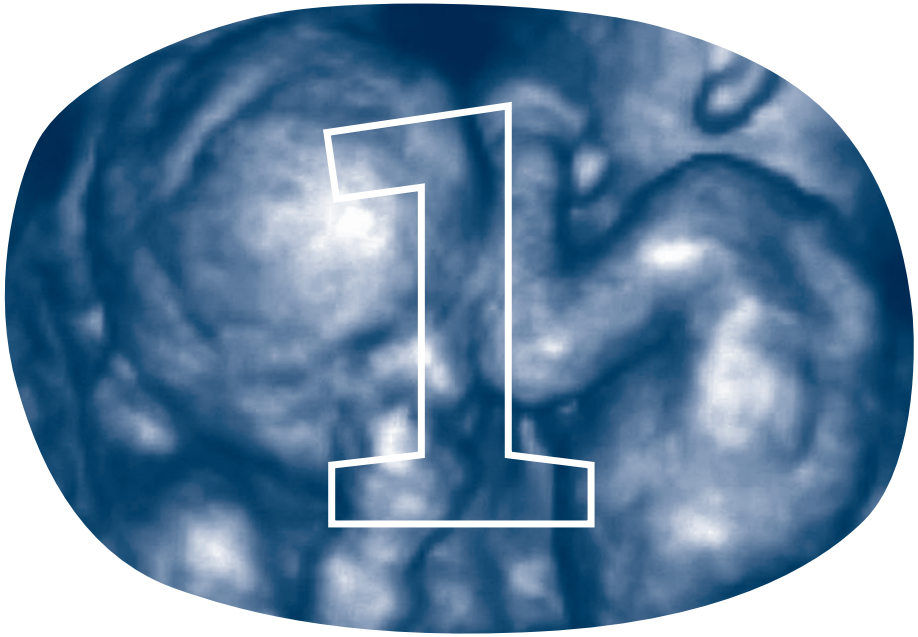

General Introduction

General Introduction

Obstetricians and other health care professionals are confronted with increasing rates of multiple pregnancies. Our national birth rate database reported in 2008 and 2009 around 3200 multiple births, predominantly twin births, representing 1.5 to 2% of all births (http://stattline.cbs.nl). Advanced maternal age at conception and the widespread use of assisted reproductive technologies (ART) are associated with the increased twinning rate. Prenatal screening for chromosomal and structural fetal anomalies has become part of the routine obstetric care in the Western World. However, studies on prenatal screening focus primarily on singleton pregnancies and reported data on twin pregnancies are scarce. In the introduction of this thesis we describe general background information on twin pregnancies concerning zygosity and chorionicity complemented by information on fetal anomalies. In this thesis options for aneuploidy and structural anomalies screening in twin pregnancies are described and in comparison with singleton pregnancies. Maternal serum and fetal ultrasound markers, both old and new in the first trimester of pregnancy are evaluated.

. . . Zygosity and chorionicity

Approximately two third of all twin pregnancies are dizygotic and one third monozygotic twins. Dizygotic (DZ = non-identical/fraternal) twins result from the fertilization of two individual oocytes. Monozygotic (MZ = identical) twins develop from one single fertilized ovum splitting into two embryos [1]. Zygosity can be determined by DNA profiling after birth or during pregnancy with invasive prenatal diagnostic testing. Zygosity cannot be determined by ultrasound during pregnancy, while chorionicity can. Chorionicity refers to the type of placentation. To determine chorionicity an ultrasound examination at 10-14 weeks of gestation is preferable. During this scan the number of placental sites, the thickness of the inter-twin membrane and presence of the lambda (λ) sign are examined. The λ-sign is seen in dichorionic pregnancies: the dividing wall between the fetuses is four layers thick and contains chorion. In a monochorionic pregnancy a T-sign is seen. Here the dividing wall is 2 layers thick and does not contain chorion [2-4]. Assessment of chorionicity before 14 weeks of gestation has a sensitivity of 89.8% and a specificity of 99.5% [5].
In general DZ twins are considered dichorionic (DC), however, contrary case reports exist [6]. DZ twins have their own placenta and fetal circulations are therefore independent of each other. During implantation the placentas may fuse in DZ twins, but the chorion stays separated. For MZ twins, chorionicity is determined on the day the fertilized ovum divides. In one third of the MZ cases splitting of the ovum occurs within three days which leads after fertilization, to the ultrasound classification of a DC twin. In two thirds of the cases splitting occurs after three days resulting in monochorionic diamniotic (MCDA) and in rare cases in monoamniotic (MCMA) twins [1].

. . . Fetal anomalies

Fetal anomalies can be either structural and/or chromosomal. A structural anomaly is defined as an unusual anatomic feature. Aneuploid is defined as an abnormal number of chromosomes. Trisomy 21 (also known as Down syndrome) is the most common chromosomal anomaly (birth prevalence approximately 1 to 2 per 1000), characterised by mental retardation and associated with congenital structural anomalies, for example congenital heart disease. The first prenatal diagnosis of trisomy 21 was reported in 1968 [7]. The life expectancy of patients with trisomy 21 is significantly lower compared with healthy individuals [8]. The prevalence of trisomy 21 affected pregnancies increases with maternal age [9;10]. Relatively common other chromosomal aneuploidies are trisomy 18 (Edwards syndrome) and trisomy 13 (Patau syndrome). Trisomy 18 has a birth prevalence of approximately 1 in 8000; trisomy 13 has a birth prevalence of approximately 1 in 17000 [11]. Both trisomies 13 and 18 are associated with multiple congenital structural anomalies and in both disorders there is a high rate of spontaneous pregnancy loss or stillbirth. Therefore, the incidence of these abnormalities is much higher in the first trimester of pregnancy than at birth. Born alive nearly all affected infants die within the first year after birth.

. . . Prenatal screening

Prenatal screening aims to identify women with a high risk for carrying a fetus with a certain anomaly. Women with a screen positive test result are offered an invasive diagnostic test, or they choose to refrain from further testing. Invasive diagnostics, e.g. chorionic villus sampling or amniocentesis provides certainty whether or not a pregnancy is affected by a chromosomal anomaly. Invasive diagnostic tests, however, carry an iatrogenic risk on fetal loss that exceeds the background fetal loss rate. Prenatal testing on trisomy 21 has become part of the routine obstetric care. Currently, in the Dutch national screening program for Down syndrome all pregnant women are informed about the possibility of prenatal screening and for pregnant women from 36 years and older are offered prenatal screening or diagnostic testing. Moreover, since January 2007 all pregnant women in the Netherlands are offered a Standard Anomaly Scan (SAS) at 18-22 weeks of gestation for detecting neural tube defects and other fetal structural anomalies [12].

. . . First trimester combined test

The current test of choice for trisomy 21 screening in the Netherlands is the first trimester combined test. This test calculates the individual risk of a woman for a trisomy 21 affected pregnancy based on maternal age, ultrasound measurement of fetal nuchal translucency (NT) and maternal serum markers, free beta human Chorionic Gonadotrophin (free β-hCG) and pregnancy associated plasma protein-A (PAPP-A). To determine maternal free β-hCG and PAPP-A distributions a venous blood sample from the mother is drawn between 9-14 weeks of gestation. In general first trimester PAPP-A is decreased and free β-hCG and NT thickness increased in trisomy 21 affected pregnancies [13]. Screening performance of the first trimester combined test in singletons is reported up to a 90% detection rate (DR) for a 5% false positive rate (FPR) [13;14].

. . . Multiple of the Median

Free β-hCG serum levels rise exponentially in the first trimester of pregnancy to a peak at approximately 10 weeks gestation and decrease subsequently. PAPP-A levels rise steadily throughout gestation, during the third trimester at increasing rate. Since levels of free β-hCG and PAPP-A vary during gestation, it is important to standardize serum levels for gestational age. The observed concentration in an individual sample of a pregnant woman is expressed as a ratio to the median value in normal pregnancies with the same gestational age in days. To achieve this a median value of each individual marker must be determined in a control population for all gestational days. Individual measurements are expressed as a Multiple of the Median (MoM) value. When the distributions of the MoM values in normal and trisomy 21 affected pregnancies are known, a likelihood ratio (LR) can be calculated by comparing the distributions. An individual risk for a trisomy 21 affected pregnancy can be calculated from the LR's (PAPP-A, free β-hCG, NT) and the a priori risk usually based on maternal age [15].

. . . Prenatal screening in twin pregnancies

Twin pregnancies are at increased risk for adverse pregnancy outcome compared with singletons. Chorionicity mediates the degree of perinatal risk in any individual multiple pregnancy. In general, monochorionic (MC) twins have a higher risk of perinatal complications compared with DC twins due to inter-twin placental vascular anastomoses which connect the two fetal circulations [16;17]. Moreover, structural fetal anomalies are more frequently seen in twins compared with singletons. In particular, in monozygotic twins congenital heart defects, neural tube defects and skeletal malformations are more frequently seen [18;19].

Fetal anomaly screening in twin pregnancies is more complex than in singletons. Fetuses can either be discordant (only one affected) or concordant (both affected) for a certain anomaly. In trisomy 21 screening both 'fetus specific' markers, i.e. NT measurements, and 'pregnancy specific', i.e. maternal biochemical markers, have to be taken into consideration. Interpretation of biochemical marker levels in twins who are discordant for a certain anomaly is difficult as altered serum levels from the affected fetus may be masked by the unaffected co-twin. Determination of the individual contribution of each fetus to the serum level is not possible.

The sensitivity of NT measurements as risk estimation for trisomy 21 in twins is similar to singletons: a 88% DR at a 7.3% FPR. However, the specificity is lower since an increased nuchal translucency is more frequently seen in chromosomally normal monochorionic twins [20]. Studies on reference values for β-hCG and PAPP-A in unaffected twins reported the MoM values approximately doubled compared with unaffected singletons [21-24].

Screening performance of the first trimester combined test in twins was reported in a statistical model to detect approximately 80% detection with a fixed 5% FPR [21]. This is confirmed by clinical data, reporting a DR 75% with a 9% FPR per pregnancy [22;25]. In 2003 Wald and colleagues introduced the 'pseudo-risk' method to incorporate both ultrasound and serum markers to produce a pregnancy-specific risk for DS screening in twin pregnancies [26]. Only recently Spencer et al. suggested to incorporate a correction factor for chorionicity for PAPP-A as monochorionic twins demonstrated lower serum levels compared with dichorionic twins [27].

Follow-up after a screen positive test result (based on the NT / combined test/ structural fetal anomaly) in twin pregnancies is more complicated compared with singletons. Several decisions have to be made: parents can decide to undergo an invasive test to investigate the fetal karyotype or can decide to accept the high risk and the outcome of the fetus. Invasive testing gives two opportunities: testing both fetuses or testing the specific fetus at risk. If both fetuses are investigated separately, the risk on miscarriage can be doubled compared with testing just one fetus. If confronted with a discordant fetal abnormality in a twin, management options depend on chorionicity. Unlike in singletons, if the other fetus is not affected, the pregnancy cannot be terminated but selective feticide has to be performed. This procedure of selective termination is dependent on chorionicity and not without risk for the healthy co-twin. Sometimes it causes preterm-labour or death of the unaffected fetus [28].

. . . Aim of this thesis

Currently in the Netherlands all pregnant women are informed on the possibility of having a screening test for Down syndrome (DS), with the first trimester combined test as policy of choice for singleton pregnancies [14;29]. Since 2009 for twin pregnancies also the use of the first trimester combined test is advocated (http://www.rivm.nl/pns/Images/landelijk%20beleid%20tweelingen%20-%20versie%202009_tcm95-62264.pdf). Currently in the VU university medical center (VUMC), Amsterdam, prenatal screening for trisomy 21 in twin pregnancies is based on NT-measurements of both fetuses instead of the first combined test. Since 2004 maternal serum of twin pregnancies has been collected prospectively. Data on NT, biochemical markers and structural anomaly screening from 2004 to 2009 were studied. In this thesis we evaluated the screening options for aneuploidy and structural anomalies in twin pregnancies, including old and new screening markers, both fetal ultrasound and maternal serum combined with screening for adverse pregnancy outcome in twin pregnancies. New screening markers, such as A Disintegrin and Metalloprotease 12s (ADAM12s) and Placental Protein 13 (PP13) are evaluated as screening markers for fetal trisomy and adverse pregnancy outcome. Moreover, the current markers from the combined test are investigated as markers for adverse pregnancy outcome. ADAM12s is the short and secreted splice form of ADAM12, a placenta-derived glycoprotein produced by trophoblasts, that is involved in fetal growth and differentiation [30]. ADAM12s was reported to be reduced in the first trimester of singleton pregnancies with trisomy 21 and could thus improve screening performance if added to the combined test [31]. Placental Protein 13 (PP13) is a small dimer protein involved in implantation and spiral artery modification [32]. Studies have reported on low serum PP13 levels and subsequent development of preeclampsia [33;34].

The following questions are addressed in this thesis:

- What are the serum distributions of the current first trimester combined test markers, free β-hCG and PAPP-A, in twin pregnancies compared with singletons? Are marker distributions different in monochorionic and dichorionic twins?
- What is the screening performance of the first trimester combined test if A Disintegrin and Metalloprotease-12s (ADAM12s) is added to the current markers in singletons

pregnancies? Is ADAM12s a potential new marker in screening for other trisomies? Can ADAM12s be applied for trisomy 21 screening to improve screening performance in twin pregnancies? Are marker distributions different in monochorionic and dichorionic twins? Is ADAM12s a new first trimester serum markers to identify adverse outcome in singletons and multiple pregnancies?

- Can discordance in nuchal translucency and first trimester biochemical serum markers in monochorionic twins be used for screening on subsequent TTTS development next to aneuploidy screening?
- What is the pregnancy outcome of twin pregnancies with discordant structural anomalies?

Outline of this thesis

In **Chapter 2** we looked at first trimester marker distributions of free β-hCG and PAPP-A in twin pregnancies compared with singletons. Differences in early first trimester free β-hCG and PAPP-A between mono- and dichorionic twins were studied.
In **Chapter 3, 4, 5** and **6** we studied the potential of A Disintegrin and Metalloprotease 12s (ADAM12s) as new serum marker for fetal aneuploidy and adverse outcome.
In **Chapter 3** we describe the results of experiments investigating processing effects, basic characteristics of the ADAM12s assay and guidelines for optimal usage of ADAM12s in standard praxis.
In **Chapter 4** we evaluated the potential of ADAM12s as an additional screening marker for aneuploidy in the first trimester combined test for singletons as part of the Dutch national Down syndrome screening program.
In **Chapter 5** we reported on the combined, never investigated before, potential of first trimester maternal serum measurements of ADAM12s and Placental Protein 13 (PP13) in the prediction of adverse pregnancy outcome in singletons pregnancies. Serum samples of pregnancies complicated by preeclampsia (PE), gestational hypertension (GH) and small for gestational age (SGA) fetuses were studied.
In **Chapter 6** we looked at first trimester marker distribution of ADAM12s in twin pregnancies compared with singletons, never reported on previously. Differences in ADAM12s levels between uncomplicated and twins complicated by SGA, GH, and PE were evaluated.
In **Chapter 7** we looked at the NT discordance in monochorionic twins as predictor for subsequent Twin-to-Twin Transfusion syndrome (TTTS) development besides the use for aneuploidy screening.
Chapter 8 describes first trimester DS markers in monochorionic twins complicated by TTTS, previously not investigated by others.
In **Chapter 9** we described the findings of Standard Anomaly Scan and discuss the management options of discordant abnormalities in twin pregnancies.
Results and perspectives are discussed in Chapter 10.

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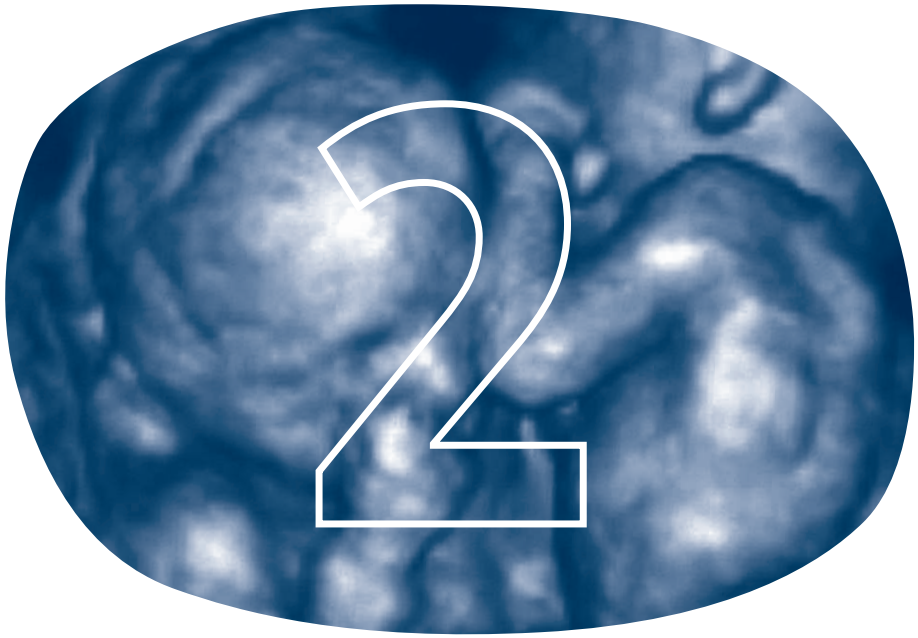

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Early first trimester free β-hCG and PAPP-A serum distributions in monochorionic and dichorionic twins

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