First trimester prenatal screening among women pregnant after IVF/ICSI

Anne Cathrine Gjerris1,*, Ann Tabor2, Anne Loft3, Michael Christiansen4, and Anja Pinborg3

1Department of Obstetrics and Gynecology, Hillerod Hospital, Dyrehavevej 29, 3400 Hillerod, Denmark 2Department of Fetal Medicine, Rigshospitalet Section 4002, Copenhagen University Hospital, 2100 Copenhagen, Denmark 3The Fertility Clinic, Rigshospitalet Section 4071, Copenhagen University Hospital, 2100 Copenhagen, Denmark 4Department of Clinical Biochemistry and Immunology, Statens Serum Institut, 2300 Copenhagen, Denmark

*Correspondence address. Tel: +45-48293707; Fax: +45-48293716; E-mail: ac@gjerris.dk

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**BACKGROUND:** Prenatal screening and diagnosis of chromosomal abnormalities especially Down’s syndrome in IVF pregnancies are complicated by higher maternal age, a high multiple pregnancy rate, a high risk of a vanishing twin and an increased risk of chromosomal abnormalities, particularly in pregnancies after ICSI. The aim of the present systematic review was to evaluate the findings of first trimester screening for chromosomal abnormalities in IVF/ICSI singleton and twin pregnancies.

**METHODS:** A systematic MESH-term search in MEDLINE using PubMed and the Cochrane Library was performed until May 2011, with no earlier date limit.

**RESULTS:** The electronic search retrieved 562 citations, 96 of which were evaluated in detail and 57 were then excluded for not meeting the selection criteria. A total of 61 articles were finally selected for review. Our analysis of the data shows that, for IVF/ICSI singletons, combined first trimester prenatal screening based on maternal age, nuchal translucency scan and biomarkers is appropriate. However, biomarkers seem to be altered, causing a higher false-positive rate, in IVF/ICSI singleton gestations. Correction factors have been developed and should be used when screening for Down’s syndrome in singleton pregnancies. With regard to IVF/ICSI twin pregnancies, biomarker values seem to be dependent on chorionicity as well as gestational age. Whether the use of a correction factor for mode of conception in the risk calculations for Down’s syndrome in twin pregnancies is valid has not been fully elucidated. In vanishing twin pregnancies with a second gestational sac with a dead fetus, first trimester screening should be based solely on the maternal age and the nuchal translucency scan as biomarkers are significantly altered in these cases.

**CONCLUSIONS:** First trimester prenatal screening after IVF/ICSI treatment requires specific precautions in both singleton and twin pregnancies.

**Key words:** chromosomal anomalies / first trimester screening / IVF/ICSI/Down’s syndrome
Introduction

As prenatal screening for chromosomal abnormalities (particularly Down’s syndrome) using maternal serum and sonographic markers has become a routine part of antenatal care in many countries, it has also become accessible to women pregnant after IVF. However, several issues complicate prenatal screening in IVF pregnancies: the mean maternal age that is higher than in women who conceive without fertility treatment, a higher multiple pregnancy and vanishing twin rate that is observed and pregnancies conceived after ICSI may have a higher rate of chromosomal abnormalities (Bonduelle et al., 2002; Gjerris et al., 2008a). With regard to prenatal diagnosis, women pregnant after IVF are less likely than women who become pregnant naturally to take up the offer of an invasive procedure (Abu-Musa et al., 2008; Gjerris et al., 2008a). This is most likely because of the 0.5–1.0% procedure-related spontaneous abortion rate associated with chorionic villus sampling or amniocentesis in singleton pregnancies (Mujezinovic and Alfrevic, 2007; Tabor et al., 2009). In twin pregnancies, the spontaneous abortion risk seems to be around twice that of singleton pregnancies (Tabor and Alfrevic, 2010).

IVF/ICSI and risk of chromosomal abnormalities

Women who become pregnant after IVF are generally older and thus more likely to carry a fetus with a chromosomal disorder, especially Down’s syndrome (Snijders et al., 1995; Meyers et al., 1997). Furthermore, reproductive failure, for example recurrent spontaneous abortion and defects of gametogenesis, is known to be associated with chromosomal aberrations (Rubio et al., 1999; Radojcic et al., 2000; Robinson et al., 2001; Bhasin 2007; Morales et al., 2008). A higher rate of chromosomal abnormalities among couples opting for fertility treatment compared with the general population has been described in several studies, 10–20% versus <1% in the general population (Radojcic et al., 2000). Additionally, an increased risk of chromosomal aberrations among IVF outcomes has been reported; however, this seems to be limited to the subgroup of ICSI offspring (Aboulghar et al., 2001; Bonduelle et al., 2002; Jozwiak et al., 2004; Shevell et al., 2005; Gjerris et al., 2008a). Three studies have found a significantly increased rate of chromosomal abnormalities in ICSI children compared with a reference group of children conceived naturally or the general population, 1.6–3.5% versus 0.0–0.9%, respectively (Aboulghar et al., 2001; Bonduelle et al., 2002; Hansen et al., 2002).

In five uncontrolled studies, the prevalence of chromosomal abnormalities in ICSI children ranged between 1.4 and 12.7% (Govaerts et al., 1998; Wennerholm et al., 2000; Samli et al., 2003; Jozwiak et al., 2004, Shevell et al., 2005). A higher rate of autosomal structural aberrations, mainly inherited from the father or de novo, and sex chromosomal abnormalities are mostly described as responsible for the difference (Bonduelle et al., 2002; Gjerris et al., 2008a; Table I).

In the first years after its introduction, most women who became pregnant after ICSI were recommended to undergo an invasive test.

Prenatal screening for chromosome abnormalities

Prenatal screening for chromosomal abnormalities seeks to identify pregnancies with a high risk of a chromosomally abnormal fetus using a combination of clinical (maternal age, prior fetus with chromosomal abnormalities), biochemical [pregnancy-associated plasma protein A (PAPP-A) and free β-hCG and ultrasound markers (nuchal translucency (NT), absence of nasal bone, tricuspid regurgitation)]. The information obtained from these markers may be combined in different ways but the most frequently used combinations are the ‘combined screening’ in first trimester with a combination of ultrasound (NT) and two serum markers which constitute the ‘double test’ (PAPP-A and free β-hCG). The blood sample should be taken at gestational age (GA) weeks 8–14. Using a risk cut-off of 1:300, the detection rate of trisomy 21 is around 70% when combining NT and maternal age for a 5% screen-positive rate, and increases to 90% when combined with the first trimester double test at the same screen-positive rate (Nicolaides, 2011). The detection rate may be increased and the false-positive rate decreased by taking the double test at an earlier GA (Kirkegaard et al., 2008). The combined screening has allowed women to make a more informed choice regarding invasive testing than based on their age alone. Among women having a risk of 1:300 or higher, women pregnant after IVF were more likely not to have an invasive procedure than women who became pregnant naturally (Wajdemann et al., 2005).

Biochemical markers

Biochemical markers (biomarkers) are serum proteins synthesized by placenta (PAPP-A, free β-hCG, hCG and unconjugated estriol (uE3) and by the fetus [alpha fetoprotein (AFP)]. Whereas a reduced maternal serum level of AFP in second trimester is caused by defective differentiation of the fetal liver in Down’s syndrome fetuses (Chen et al., 1997), the changes in the levels of placenta-derived proteins have not been explained. However, studies comparing the synthesis of placental proteins in cyto- and syncytiotrophoblasts in Down’s syndrome and normal placentae have demonstrated that the differentiation from cyto- to syncytiotrophoblast is impaired in Down’s syndrome placentae, followed by an alteration in the synthesis of placenta-derived proteins (Frendo et al., 2000).

The free β-hCG has long been used as a chemical marker for early pregnancy and it has been stated that the free β-subunit has a better predictive value in screening Down’s syndrome, although this has not been confirmed in all studies (Spencer, 1991; Krantz et al., 1996). Free β-hCG is synthesized by the trophoblast and is essential for the maintenance of pregnancy. The level of hCG in early pregnancy is assumed to represent the mass of syncytial trophoblast (Almog et al., 2011). The concentration of free β-hCG increases until gestational week 10 and then decreases towards term (Cole, 1997). Free β-hCG is measured at GA weeks 8–14 for Down’s syndrome screening. In first trimester of pregnancy, high levels of free β-hCG are associated with Down’s syndrome, and in the second trimester with poor obstetric outcome (Spencer, 2000a; Dugoff et al., 2005). The difference between the levels of β-hCG in affected and unaffected pregnancies increases with advancing GA (Spencer et al., 2003).

The level of free β-hCG is lower when comparing first trimester IVF with natural pregnancies (Zegers-Hochschild et al., 1994; Almog et al., 2011) but in the second trimester free β-hCG is higher in IVF pregnancies (Barkai et al., 1996; Wald et al., 1999; Bar-Hava et al., 2001).

The decreased level of PAPP-A seen in Down’s syndrome pregnancies represents a general depression of the insulin-like growth factor axis in Down’s syndrome pregnancies (Giudice et al., 2002; Santolaya-
pregnant after IVF or ICSI with a focus on first trimester-combined 
surrounding first trimester screening and diagnosis among women 
in IVF pregnancies and is not described in this review. CRL.

with GA, it is essential to correctly measure not only NT but also 
should be measured at 11–13 weeks and 6 days, corresponding to 
formations and genetic syndromes (Nicolaides, 2011). Fetal NT 
other chromosomal aneuploidies, as well as with a range of fetal mal-
Fetal NT thickness is associated with the risk of Down’s syndrome and 
other aspects of the fertility treatment which may affect the embryo, 
early pregnancy (Dumoulin et al., 2010). Hence, maybe the natural cycle concept in FET cycles

IVF/ICSI is normally performed after controlled ovarian hyperstimu-
lation, which results in marked endocrine changes related to matu-
rature of multiple follicles and later development of multiple corpora 
lutea. These endocrine changes may have a negative effect on implant-
ation and early pregnancy, including changes in the level of biomarkers 
for genetic diagnostic testing. In contrast to fresh IVF cycles, frozen 
embryo transfer (FET) is usually performed in natural or mildly stimu-
lated cycles, which may affect the level of first trimester serum 
markers differently. Two recent studies have shown that singletons 
born after FET have a higher mean birthweight than those after 
IVF/ICSI with fresh embryo transfer (Shih et al., 2008; Pinborg et 
Al., 2010). Hence, maybe the natural cycle concept in FET cycles 
is favourable for embryo development, implantation and early placen-
tation. Mode of fertilization, culture media and culture conditions are 

Nuchal translucency 
Fetal NT thickness is associated with the risk of Down’s syndrome and 
other chromosomal aneuploidies, as well as with a range of fetal mal-
formations and genetic syndromes (Nicolaides, 2011). Fetal NT 
should be measured at 11–13 weeks and 6 days, corresponding to 
a crown-rump length (CRL) of 45–84 mm. As NT thickness increases 
with GA, it is essential to correctly measure not only NT but also 
CRL.

The efficiency of other ultrasound markers has not been evaluated in 
IVF pregnancies and is not described in this review.

The aim of the current systematic review was to explore the issues 
surrounding first trimester screening and diagnosis among women 
pregnant after IVF or ICSI with a focus on first trimester-combined 
screening in singleton and twin pregnancies. Prenatal screening in preg-
nancies conceived after intrauterine insemination and screening for 
structural malformations is not covered in this review.

Methods

We performed a systematic literature search in MEDLINE using PubMed 
and the Cochrane Library. The following searches were performed using the 
following combination of MESH-terms: ‘Prenatal diagnosis AND Re-
productive Techniques, assisted’; ‘First trimester pregnancy AND Preg-
nancy Proteins AND Reproductive Techniques, assisted’; ‘Twins AND 
Prenatal diagnosis AND Reproductive Techniques, assisted’; and ‘Twins AND 
Pregnancy Proteins’. If one search revealed more than 1000 citations 
the MESH terms were restricted to ‘major’. The search was performed up 
to May 2011 with no lower date limit. In addition, abstracts books for the 
congresses of the International Society of Ultrasound in Obstetrics and 
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last 5 years were searched, finding one relevant citation. One main reviewer 
performed the search for each specific section and the other authors 
subsequently checked the included articles. Reference lists of all primary 
and review articles were reviewed for relevant citations. Articles in lan-
guages other than English were excluded and only IVF, ICSI and FET treat-
ments were included.

Results

A flowchart of the process to obtain the relevant citations is shown in 
Fig. 1 and the results are based on the 61 selected citations.

Prenatal screening and dating of GA after 
IVF/ICSI

Determination of GA is crucial in first trimester risk assessment. An 
ultrasound measurement of CRL together with validated algorithms 
for the conversion into GA is routinely used for GA dating at the 
NT scan. In pregnancies conceived after IVF/ICSI, an exact day of con-
ception and thereby an exact measure for GA are available. However, 
early growth and development of the fetus and/or placenta after 
IVF/ICSI might be altered (Naaktgeboren et al., 1986; Grinsted and 
Avery, 1996; Ahlborg et al., 2006). If the secretory function of the

Table I Abnormal karyotypes in ICSI pregnancies found by prenatal invasive testing.

<table>
<thead>
<tr>
<th>Studies</th>
<th>Woman having invasive testing (n)</th>
<th>Abnormal karyotype (%)</th>
<th>Mean maternal age (years)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total</td>
<td>Autosomal structural</td>
<td>Sex</td>
</tr>
<tr>
<td>Van Opstal et al. (1997)</td>
<td>71</td>
<td>12.7</td>
<td>1.4</td>
</tr>
<tr>
<td>Govaerts et al. (1998)</td>
<td>101</td>
<td>4.0</td>
<td></td>
</tr>
<tr>
<td>Wennemerholm et al. (2000)</td>
<td>149</td>
<td>2.7</td>
<td>2.0</td>
</tr>
<tr>
<td>Bonduelle et al. (2002)</td>
<td>1586</td>
<td>3.0</td>
<td>1.8</td>
</tr>
<tr>
<td>Samli et al. (2003)</td>
<td>142</td>
<td>4.2</td>
<td>0.6</td>
</tr>
<tr>
<td>Jozwiak et al. (2004)</td>
<td>1136</td>
<td>1.4</td>
<td>0.6</td>
</tr>
<tr>
<td>Gjerris et al. (2008a)</td>
<td>556</td>
<td>4.3</td>
<td>2.3</td>
</tr>
</tbody>
</table>

Indication for karyotyping was primarily ICSI treatment.

Forgas et al., 2004). As IGFs are involved in trophoblast invasion, 
changes in the bioavailability of these hormones may be related to 
the increased incidence of pregnancy complications associated with 
Downs’ syndrome pregnancies. The decrease in maternal serum 
levels of PAPP-A in Down’s syndrome pregnancies is not specific, as 
low levels of PAPP-A are also seen in pregnancies with pre-eclampsia 
and intrauterine growth retardation (Spencer, 2000a; Dugoff et 
Al., 2005; Pihl et al., 2008).

IVF/ICSI is normally performed after controlled ovarian hyperstimu-
lation, which results in marked endocrine changes related to matur-
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lutea. These endocrine changes may have a negative effect on implant-
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early growth and development of the fetus and/or placenta after 
IVF/ICSI might be altered (Naaktgeboren et al., 1986; Grinsted and 
Avery, 1996; Ahlborg et al., 2006). If the secretory function of the
placenta or the size of the fetus develops differently with time in IVF/ICSI pregnancies compared with pregnancies conceived naturally, this may influence the calculation of multiples of the median (MoM) of biochemical and ultrasound prenatal screening markers and, in consequence, affect the screening performance by increasing the false-positive rate in IVF/ICSI pregnancies. One study has evaluated the significance of calculating GA by either CRL or day of oocyte aspiration in relation to first trimester combined screening. The conclusion was that day of oocyte aspiration and CRL were equivalent when calculating GA for first trimester serum screening. However, whether the day of oocyte aspiration is the correct method of GA dating for other purposes (e.g. estimated date of delivery) in IVF/ICSI pregnancies is still unknown (Gjerris et al., 2008b).

First trimester screening in singleton pregnancies after IVF/ICSI

Biochemical markers

Several studies from the 1990s showed that the second trimester serum triple test markers were significantly altered among women who conceived after IVF, with higher values of β-hCG and lower values of AFP and uE3, leading to increased false-positive rates in IVF versus non-IVF pregnancies. Thus, it was recommended not to use the triple test in women who had conceived after IVF/ICSI (Barkai et al., 1996; Wald et al., 1999; Bar-Hava et al., 2001).

The most consistent finding for first trimester serum markers is a decreased level of PAPP-A to around 0.8 MoM in both IVF and ICSI pregnancies after fresh embryo transfer (Tables II and III; Liao et al., 2001; Hui et al., 2005a; Tul and Novak-Antolic, 2006; Anckaert et al., 2008; Amor et al., 2009; Gjerris et al., 2009a,b; Bender et al., 2010; Engels et al., 2010; Casanova et al., 2011). In FET pregnancies, PAPP-A was only decreased in two out of five studies (Table IV; Hui et al., 2005a; Anckaert et al., 2008; Amor et al., 2009; Gjerris et al., 2009a,b; Matilainen et al., 2011). In the large Australian study, only FET cycles accompanied by hormone treatment and not natural cycle FET showed a decreased PAPP-A level (0.78 versus 0.99 MoM, P < 0.001; Amor et al., 2009), while in the smaller American study only ICSI-FET, but not IVF-FET, was associated with a decreased PAPP-A level (Table IV; Hui et al., 2005a).

Tul and Novak-Antolic (2006) reported an inverse association between the number of aspirated oocytes and PAPP-A MoM values. It was hypothesized that the number of oocytes retrieved reflected the number of corpora lutea in pregnancies, supported by their other finding that Inhibin A was increased with decreasing PAPP-A and increasing number of retrieved oocytes. Inhibin A is secreted by corpora lutea and it was suggested that Inhibin A inhibits the secretion of PAPP-A (Tul and Novak-Antolic, 2006).

These findings might lead to the conclusion that ovarian stimulation plays an important role in the biological cascade leading to decreased levels of PAPP-A in IVF pregnancies. However, a recent study found that in pregnancies conceived naturally with time-to-pregnancy (TTP) > 2 years the levels of PAPP-A were similar to levels in IVF pregnancies (Ranta et al., 2010). In women with TTP > 2 years, the mean MoM PAPP-A was 0.83 compared with 0.84 in the IVF group and 1.03 in the reference group with TTP < 1 year (P < 0.01). Hence, it seems likely that the changes in PAPP-A levels in IVF pregnancies are caused by several factors, which include subfertility and hormone stimulation.

Three studies have found increased β-hCG levels in the first trimester after IVF/ICSI (Tables II and III; Liao et al., 2001; Ghisoni et al., 2003; Bender et al., 2010). This might be expected as β-hCG was significantly elevated in the second trimester triple test in IVF/ICSI pregnancies, as referred to in the beginning of this section. On the other hand, very early pregnancy levels of β-hCG were found to be decreased (Zegers-Hochschild et al., 1994; Almog et al., 2011). This is in accordance with several other studies, which have found that first trimester levels of β-hCG are unaltered or lower in IVF singleton pregnancies (Wojdemann et al., 2001; Orlandi et al., 2002; Maymon and Shulman et al., 2004; Hui et al., 2005a; Lambert-Messerlian et al., 2006; Tul and Novak-Antolic, 2006; Anckaert et al., 2008; Amor et al., 2009; Gjerris et al., 2009a,b; Engels et al., 2010). The
explanation for this paradox may be that in those studies finding elevated β-hCG, blood samples were taken at GA 11–13 weeks, in contrast to studies with similar β-hCG levels in IVF and non-IVF pregnancies, where blood samples were taken earlier, in gestational week 8. Further, Gjerris et al. (2009a,b) found that in the IVF/ICSI group, β-hCG MoM values increased significantly with increasing GA, which was not the case for the control group, suggesting different serum marker levels over time among women pregnant after IVF/ICSI.

<table>
<thead>
<tr>
<th>Table II</th>
<th>First trimester biochemical and ultrasonic screening markers in pregnancies after IVF.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reference</td>
<td>Number of cases (n)</td>
</tr>
<tr>
<td>Liao et al. (2001)</td>
<td>220</td>
</tr>
<tr>
<td>Wædemann et al. (2001)</td>
<td>47</td>
</tr>
<tr>
<td>Orlandi et al. (2002)</td>
<td>32</td>
</tr>
<tr>
<td>Ghisoni et al. (2003)</td>
<td>50</td>
</tr>
<tr>
<td>Maymon and Shulman (2004)</td>
<td>99</td>
</tr>
<tr>
<td>Hui et al. (2005a)</td>
<td>92</td>
</tr>
<tr>
<td>Hui et al. (2005b)</td>
<td>119</td>
</tr>
<tr>
<td>Tul and Novak-Antolic (2006)</td>
<td>130</td>
</tr>
<tr>
<td>Lambert-Messerlian et al. (2006)</td>
<td>277</td>
</tr>
<tr>
<td>Anckaert et al. (2008)</td>
<td>59</td>
</tr>
<tr>
<td>Gjerris et al. (2009a,b)</td>
<td>512</td>
</tr>
<tr>
<td>Amor et al. (2009)</td>
<td>513</td>
</tr>
<tr>
<td>Engels et al. (2010)</td>
<td>203</td>
</tr>
<tr>
<td>Bender et al., 2010</td>
<td>110</td>
</tr>
<tr>
<td>Matilainen et al. (2011)</td>
<td>176&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

PAPP-A, pregnancy associated plasma protein-A; NT, nuchal translucency; MoM, multiple of the median.
<sup>a</sup>Significantly lower compared with the control group.
<sup>b</sup>Significantly higher compared with the control group.
<sup>c</sup>IVF and ICSI jointly.

<table>
<thead>
<tr>
<th>Table III</th>
<th>First trimester biochemical and ultrasonic screening markers in pregnancies after ICSI.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reference</td>
<td>Number of cases (n)</td>
</tr>
<tr>
<td>Liao et al. (2001)</td>
<td>30</td>
</tr>
<tr>
<td>Orlandi et al. (2002)</td>
<td>42</td>
</tr>
<tr>
<td>Ghisoni et al. (2003)</td>
<td>92</td>
</tr>
<tr>
<td>Hui et al. (2005a)</td>
<td>57</td>
</tr>
<tr>
<td>Hui et al. (2005b)</td>
<td>81</td>
</tr>
<tr>
<td>Tul and Novak-Antolic (2006)</td>
<td>54</td>
</tr>
<tr>
<td>Anckaert et al. (2008)</td>
<td>163</td>
</tr>
<tr>
<td>Gjerris et al. (2009a,b)</td>
<td>396</td>
</tr>
<tr>
<td>Amor et al. (2009)</td>
<td>833</td>
</tr>
<tr>
<td>Engels et al. (2010)</td>
<td>192</td>
</tr>
<tr>
<td>Bender et al. (2010)</td>
<td>331</td>
</tr>
<tr>
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</tr>
</tbody>
</table>

<sup>a</sup>Significantly lower compared with the control group.
<sup>b</sup>Significantly higher compared with the control group.
<sup>c</sup>IVF and ICSI jointly.

Nuchal translucency
The majority of studies found no difference in the NT MoM mean value between IVF and non-IVF pregnancies (Tables II and III). Two studies, however, found thicker NT among IVF/ICSI fetuses (Maymon and Shulman, 2004; Hui et al., 2005b), while another two studies found thinner NT in IVF pregnancies (Gjerris et al., 2009a,b; Engels et al., 2010). For FET pregnancies a similar NT as the control groups was found (Table IV). There are no obvious biological
Table IV First trimester biochemical and ultrasonic screening markers in pregnancies after FET.

<table>
<thead>
<tr>
<th>Study</th>
<th>Number of cases (n)</th>
<th>PAPP-A (MoM)</th>
<th>Free β-hCG (MoM)</th>
<th>NT (MoM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hui et al. (2005a)</td>
<td>IVF 54</td>
<td>0.95</td>
<td>1.21</td>
<td></td>
</tr>
<tr>
<td></td>
<td>ICSI 31</td>
<td><strong>0.66</strong></td>
<td>0.96</td>
<td></td>
</tr>
<tr>
<td>Hui et al. (2005b)</td>
<td>IVF-FET 62</td>
<td>1.09</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>ICSI-FET 39</td>
<td>1.04</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gjerris et al. (2009a,b)</td>
<td>85</td>
<td>1.03</td>
<td>1.00</td>
<td>0.94</td>
</tr>
<tr>
<td>Amor et al. (2009)</td>
<td>With hormone treatment 118</td>
<td><strong>0.78</strong></td>
<td>0.98</td>
<td>0.93</td>
</tr>
<tr>
<td></td>
<td>Without hormone treatment 445</td>
<td>0.99</td>
<td>1.06</td>
<td>0.92</td>
</tr>
<tr>
<td>Matilainen et al. (2011)</td>
<td>87</td>
<td>0.78</td>
<td>0.94</td>
<td>1.00</td>
</tr>
</tbody>
</table>

FET, frozen embryo transfer.
*aSignificantly lower compared with control group.

explanations for altered NT in IVF/ICSI pregnancies and the findings of significant alterations might be random.

**Combined screening in singleton pregnancy**

As a consequence of the decreased level of PAPP-A, the false-positive rate for combined screening in IVF/ICSI pregnancies has been increased from 4.7 to 15.9% compared with 3.1 to 8.6% in non-IVF groups (Liao et al., 2001; Wejdemann et al., 2001; Orlandi et al., 2002; Ghisoni et al., 2003; Maymon and Shulman, 2004; Amor et al., 2009; Gjerris et al., 2009a,b; Engels et al., 2010). This increase can, to a certain extent, be explained by the higher maternal age among women who underwent IVF/ICSI to become pregnant; however, even in studies using age-matched controls and in studies correcting for maternal age the false-positive rate is persistently increased after IVF/ICSI (Liao et al., 2001; Amor et al., 2009; Gjerris et al., 2009a,b; Engels et al., 2010).

It has been suggested that the reason for the lower levels of PAPP-A is the documented increased risk of adverse obstetric outcome among IVF/ICSI pregnancies, as adverse obstetric outcome per se is associated with decreased PAPP-A. Nevertheless, Engels et al. (2010) excluded all cases with adverse obstetric outcome, and the PAPP-A values in IVF pregnancies remained low. Further, Bender et al. (2010) analysed 110 IVF, 331 ICSI and 1431 pregnancies after natural conception for the relationship of first-trimester screening markers and small-for-gestational age and number of embryos transferred. In analyses only including neonates with birthweight appropriate for GA, PAPP-A values remained significantly lower in IVF versus non-IVF (Bender et al., 2010). No relationship between serum marker values and the number of embryos transferred was observed.

For IVF/ICSI singleton pregnancies, the use of combined first trimester prenatal screening, including maternal age, NT scan and biomarkers, is supported by the data. Biomarkers, however, seem to be altered causing a higher false-positive rate. Therefore, it is appropriate to correct for mode of conception and hence most computer software for prenatal risk calculation is programmed to correct IVF/ICSI with a predefined correction factor. Still, caution must be taken as the aetiology of the infertility, the treatment modality and the response to the treatment may cause different changes in the screening markers. For the subgroup of pregnancies conceived after FET, it is still uncertain whether or not to correct for mode of conception as data are limited.

**First trimester screening in twin pregnancies after IVF/ICSI**

In twin pregnancies conceived naturally, about one-third are monzygotic and two-thirds are dizygotic. Dizygotic twins are always dichorionic, while about one out of four monzygotic twins are dichorionic. Zygosity relates to the fetal DNA and determines whether the fetuses are concordant (both affected) or discordant (only one affected) for a certain anomaly. Chorionicity, which refers to the type of placenta, can be accurately determined by first-trimester ultrasound examining the number of placental sites, the thickness of the inter-twin membrane, the lambda and the T signs (Monteagudo et al., 1994; Sepulveda et al., 1996; Lee et al., 2006). The vast majority of IVF twin pregnancies are the result of the transfer and implantation of two embryos and are therefore dizygotic and dichorionic (98–99%). Nevertheless, the rate of monochorionic twinning is still higher in IVF gestations than after natural conceptions (Wenstrom et al., 1993). In particular, advanced assisted reproduction technologies, such as ICSI, assisted hatching and blastocyst culture, increase the risk of monochorionic twinning (Skiafas et al., 2008) with a 10–15% risk of twin-to-twin transfusion syndrome (Bermudez et al., 2002).

Prenatal screening of IVF/ICSI twin pregnancies faces two problems. First, biochemical tests in dizygotic twins are limited by the masking effect of the normal co-twin and the difficulty in identifying the abnormal twin (Cuckle, 1998; Maymon and Jauniaux, 2002). The second obstacle is that biochemical marker levels differ between IVF/ICSI and pregnancies conceived naturally.

**Biochemical markers**

The maternal first-trimester serum levels of PAPP-A and free β-hCG are approximately twice the concentration in twin compared with singleton pregnancies (Raty et al., 2000; Spencer, 2000b; Niemimaa et al., 2002; Spencer and Nicolaides, 2003; Mashiach et al., 2004; Goncé et al., 2005; Wejdemann et al., 2006; Spencer et al., 2008; Zheng et al., 2010).

Earlier smaller series found that chorionicity did not influence the levels of PAPP-A and β-hCG (Spencer 2001a,b; Niemimaa et al., 2002; Goncé et al., 2005; Wejdemann et al., 2006) but in two recent larger studies both PAPP-A and β-hCG were significantly dependent on chorionicity, the latest study including >5000 twin pregnancies (Linskens et al., 2009; Madsen et al., 2011).
A Finnish study with 145 twin pregnancies revealed that in IVF twin pregnancies β-hCG levels were higher than in natural twin pregnancies, whereas no significant difference was found in the AFP level (Raty et al., 2000). The authors concluded that the higher β-hCG levels in IVF twin pregnancies should be considered in screening Down’s syndrome to avoid higher false-positive rates (Raty et al., 2000). Later studies showed no significant differences in biomarkers between IVF and natural twin pregnancies (Orlandi et al., 2002; Goncé et al., 2005; Linskens et al., 2009). However, a recent larger study found significantly higher levels of PAPP-A in IVF pregnancies, while levels of β-hCG were unaffected by mode of conception (Madsen et al., 2011). From the current literature, it is uncertain how mode of conception affects biochemical marker levels in IVF twin pregnancies.

Nuchal translucency

Reports have shown that the detection rate for Down’s syndrome with the use of maternal age and NT in twin and singleton pregnancies is similar, although with a higher false-positive rate in twin pregnancies (Pandya et al., 1995; Sebire et al., 1996). In twin pregnancies conceived naturally, the NT is affected by chorionicity, as an increased NT was found in chromosomally normal fetuses from monochorionic, compared with dichorionic, pregnancies (Sebire et al., 1996; Monni et al., 2000).

NT values for fetuses in subsequent pregnancies from the same woman do not correlate (Spencer 2001a, b) but based on 181 twin pregnancies (31 mono- and 150 dichorionic) with a normal outcome, a Danish group showed that NT measurements are highly correlated in both mono- and dichorionic twin pairs (Wojdemann et al., 2006). This indicates that the NT is influenced by environmental, placental, maternal or other factors specific for the actual pregnancy. The authors advocated that because NT correlates within a twin pair, the NT measurement of the co-twin should be used as an additional risk marker in dichorionic dizygotic twin pairs. Hence, a large NT in one twin should reduce the risk associated with a large NT in the other twin. The precise gain in performance by including the NT of the other twin in dizygotic dichorionic twin pregnancies still needs to be ascertained. A recent US study confirmed that there is a correlation between the NT values in twin fetuses and described a detailed method to calculate the risk of Down’s syndrome in a twin fetus using its own NT and that of the co-twin (Cuckle and Maymon, 2010). The authors underline the importance of using this correlation coefficient between NT values in twin fetuses, as these values are not independent.

A retrospective study including only dichorionic twins showed that NT in dichorionic IVF twins was similar to the NT in non-IVF twins in contrast to their findings for IVF singletons where NT was significantly thicker in IVF singletons (Hui et al., 2005b). The authors concluded that mode of conception appears to influence singleton and twin pregnancies differently (Hui et al., 2006). The sample size was, however, limited (comparing 27 IVF and 19 naturally conceived twin pairs) and as an insignificant reduction in NT in IVF compared with naturally conceived twins was revealed, a type two error cannot be excluded (Hui et al., 2006). An earlier Italian study found a small but insignificant decrease in NT in IVF compared with twins conceived naturally (Orlandi et al., 2002).

Based on the existing evidence it is difficult to draw any firm conclusion on the NT thickness in IVF twins compared with twins conceived naturally. Although NT values in twin pairs are not fully independent, NT can still be used as a first-trimester screening marker for chromosomal abnormality in IVF twin pregnancies, if the NT of the co-twin is taken into consideration.

Combined screening in twin pregnancy

No data are available on combined first-trimester screening in twins conceived naturally versus after IVF. Studies have shown, however, that in order to overcome the problem with serological markers in twin pregnancies a ‘pseudo-risk’ for each twin in a dizygotic twin-pair and a combined risk in monozygous twins can be calculated (Spencer et al., 1994; Wald et al., 2003; Wojdemann et al., 2006). Visual identification of the affected fetus with the help of the NT is essential to perform this approximation (Nicolaides, 2004) but screening performance for Down’s syndrome in twin pregnancies is still reduced compared with singleton pregnancies (Cuckle, 1998). It has been very difficult to develop risk algorithms for twin pregnancies (Spencer, 2000b; Niemimaa et al., 2002; Spencer and Nicolaides, 2003). Data from Down’s syndrome twin pregnancies are scarce and from assays which are not directly comparable (Bersinger et al., 2003; Spencer and Nicolaides, 2003). One theoretical model suggested a 70% Down’s syndrome detection rate with a 5% false-positive rate in dichorionic twin pregnancies (Wald et al., 2003).

In a more recent US study of 535 twin sets with 6.7% monochorionic twin pregnancies and 53.3% achieved by IVF, seven Down’s syndrome fetuses were identified in six pregnancies (Chasen et al., 2007). In their study maternal age alone was associated with a 33% detection rate for trisomy 18 or 21. The addition of NT increased the sensitivity to 83%, while combining age, NT and biomarkers (free or total β-hCG and PAPP-A) increased sensitivity to 100% with a 5% false-positive rate and with a cut-off of 1:300, and addition of biomarkers lowered the false positive rate (Chasen et al., 2007). Consistently, another study on 100 twin pregnancies with three Down’s syndrome cases revealed that the addition of biochemistry did not affect the detection rate but reduced the false-positive rate (Goncé et al., 2005). In a study including 41 Down’s syndrome cases, including biochemical markers in the risk calculation increased the detection rate from 78 to 90% and reduced the false-positive rate from 8.0 to 5.9% (Madsen et al., 2011).

As stated above, to our knowledge there are no studies available on combined first-trimester screening in naturally conceived twins versus those conceived after IVF. Biochemical screening markers should not be used in IVF twin pregnancies until risk calculation algorithms based on a sufficiently large population of Down’s syndrome fetuses in IVF twin pregnancies are available. Until then, first trimester screening in IVF twin pregnancies should be based on maternal age and NT.

Pregnancies with a vanishing twin

Studies have shown that 12–30% of IVF/ICSI twin pregnancies recognized by early ultrasound in pregnancy weeks 7–8 are spontaneously reduced to singleton pregnancies, known as the ‘vanishing twin’ phenomenon (Pinborg et al., 2005; Chasen et al., 2006). One study of 41 cases with a vanishing twin, including both IVF/ICSI and naturally conceived pregnancies, showed no difference in the levels of either first
trimester PAPP-A and free β-hCG serum markers between vanishing twin and singleton pregnancies. Only if the reduction happened within 4 weeks of taking the blood sample, both PAPP-A and free β-hCG were significantly increased in pregnancies with a vanishing twin compared with singleton pregnancies (1.79 MoM versus 1.18 MoM and 1.28 versus 0.96, respectively; Chasen et al., 2006). A Danish study with 56 cases of IVF pregnancies with a vanishing twin and a control group of 897 IVF singleton pregnancies showed no significant differences in the mean MoM free β-hCG and PAPP-A between pregnancies with an early (gestational week <9) or late vanishing twin (gestational weeks 9–13) or singleton pregnancies (0.98, 1.13 and 0.95 for free β-hCG and 0.84, 0.80 and 0.74 for PAPP-A, respectively). Likewise, no difference was seen for NT measurements. However, the number of late vanishing twins was very small and both PAPP-A and free β-hCG serum markers showed a tendency to be increased (Gjerris et al., 2009,a,b). A recently published retrospective analysis of β-hCG and PAPP-A levels in 270 women with a normal singleton fetus with ultrasound evidence of a vanishing twin pregnancy compared three groups: 76 women with a second empty gestational sac, 194 women with a second gestational sac containing a dead fetus with a measurable CRL and 1360 matched singleton pregnancies (Spencer et al., 2010). In women with a second empty gestational sac, screening marker values were similar to the singleton pregnancies, however in the group with a second gestational sac with a dead fetus with measurable CRL, there was a significantly increased median PAPP-A, while beta-hCG was unaffected. Modelling this bias in PAPP-A MoM, the detection rate for trisomy 21 would fall from 85 to 75%. The authors concluded that in the presence of a dead fetus with a measurable CRL, the first trimester screening marker analysis may lead to errors in risk estimation. Hence, their advice was to restrict screening to the use of NT alone (Spencer et al., 2010).

Based on the largest study we consider that for first trimester screening in IVF pregnancies with a vanishing twin, verified by an empty gestational sac, biomarkers can be used but in pregnancies with a vanishing twin, verified by the presence of a dead fetus with a measurable CRL, screening should be based on maternal age and NT.

Concluding remarks

First trimester combined prenatal screening is a non-invasive screening method, which is applicable to IVF/ICSI pregnancies. However, precautions should be taken as biochemical markers are affected by the mode of conception causing a higher false-positive rate in IVF/ICSI singleton pregnancies. In IVF/ICSI twin pregnancies, biochemical marker levels differ from those twin pregnancies which are conceived naturally, and further more chorionicity and GA also affect biochemical marker levels, resulting in a need for intricate correction algorithms.

As the aetiology of infertility, fertility treatment protocols, the individual response to the treatment as well as GA and twin pregnancy all influence the levels of screening markers in different ways, it is difficult to arrive at the optimal correction factor/method. More research on larger series of IVF pregnancies is highly warranted to develop exact correction factors for fresh and frozen IVF and ICSI treatment, specific for singleton and twin pregnancies. Finally, it remains to be proved that applying correction factors in IVF/ICSI pregnancies results in a better screening performance. Implementing such correction factors in daily practice, where screening is performed by many practitioners is complicated and requires individual knowledge of fertility treatment methods in all those performing the ultrasound scans and the risk calculations.

Authors’ roles

A.C.G. designed the study, selected articles and extracted data and wrote the major part of the manuscript. A.L., A.T. and M.C. contributed to the analysis and interpretation of the data, wrote a minor part of the manuscript and critically revised the manuscript. A.P. contributed to study conception and design, contributed to the analysis and interpretation of the data, wrote a part of the manuscript and critically revised the manuscript for important intellectual content. All the authors approved the final version of the manuscript.

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Conflict of interest

None declared.

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