ORIGINAL ARTICLE

Increased time-to-pregnancy and first trimester Down’s syndrome screening

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BACKGROUND: Time-to-pregnancy (TTP) is a clinical tool used to measure uterine receptivity and a couples’ fertility in spontaneously conceived pregnancies. The objective of this study was to examine the effects of TTP on first trimester Down’s syndrome (DS) markers in spontaneous, chromosomally normal pregnancies and to compare the results to those in IVF pregnancies.

METHODS: A case–control study was conducted amongst patients attending a university hospital in Finland. During 2005–2007 data on pregnant women in Kuopio, with singleton pregnancies, routinely collected by the Department of Obstetrics and Gynaecology of Kuopio University Hospital and Eastern Finland Laboratory Centre were compiled. The data comprised information gathered in first trimester DS screening [age of the mother, serum hCG free beta subunit (fβ-hCG) and pregnancy-associated plasma protein A (S-PAPP-A) levels and the nuchal translucency (NT) of the fetus], body mass index, method of conception [spontaneous or in vitro fertilization (IVF)], TTP (in spontaneous pregnancies), maternal chronic diseases, smoking habits of the mother, outcome of the pregnancy and prior pregnancy complications. Spontaneous pregnancies were classified into three groups by TTP: 0–12 months (the reference group, N = 1164), 13–24 months (N = 112) and /C2125 months (N = 70). Screening data from IVF pregnancies (N = 39) were collected for comparison. The size of the total study population was 1385.

RESULTS: The median/geometric mean multiple of median (MOM) of S-PAPP-A was significantly lower (P < 0.01) in women with a TTP over 25 months (0.89/0.83 MOM) and in the IVF group (0.95/0.84 MOM) compared with the reference group (1.01/1.03 MOM). However, first trimester S-fβ-hCG and NT MOMs were not statistically different between the study groups. Consequently, the proportion of DS screening positives were classified into three groups by TTP: 0–12 months (the reference group, N = 1164), 13–24 months (N = 112) and /C2125 months (N = 70). Screening data from IVF pregnancies (N = 39) were collected for comparison. The size of the total study population was 1385.

CONCLUSIONS: A TTP of over 2 years altered the levels of DS screening serum markers to levels similar to those observed in IVF pregnancies, with a decrease in PAPP-A levels compared with the reference group. These results raise the possibility that such changes could be related to subfertility rather than to the use of assisted reproductive technology.

Key words: Down’s syndrome / screening / S-PAPP-A / subfertility / time-to-pregnancy

Introduction

Waiting time-to-pregnancy (TTP), measured from the start of attempts to become pregnant to the observation of an actual pregnancy, is a clinical tool used to measure uterine receptivity and couples’ fertility in spontaneously conceived pregnancies. Extended TTP, defined in the present study as more than 2 years, is probably an indication of subfertility in couples whose intrauterine environment is similar to that of those conceiving after assisted reproduction but without the confounding effect of assisted reproduction. It was therefore hypothesized that subfertility (manifesting as a prolonged TTP) may also have an effect on placental function due to alterations in as yet unknown mechanism. Pregnancy-associated plasma protein A (PAPP-A) and hCG free beta subunit (fβ-hCG) are both produced by the placenta, thus it was speculated that the TTP may be linked to the production of these markers in the first trimester.

Levels of serum markers assessed in first trimester Down’s syndrome (DS) screening have been reported to be altered in in vitro fertilization (IVF) pregnancies, although the frequency of trisomy 21 is virtually unaffected by the use of assisted reproduction (Liao et al., 2001; Hui, et al., 2006a, b; Gjerris et al., 2009a, b). The multiple of medians (MOMs) of these markers are gestation-specific and the median fβ-hCG MOM in chromosomally normal IVF pregnancies has been shown to range from 0.84 to 1.21, although reported
median PAPP-A levels in these pregnancies have ranged from 0.75 MOM to no different to those in spontaneously conceived pregnancies (Liao et al., 2001; Orlandi et al., 2002; Ghisoni et al., 2003; Tul, Novak-Antolic, 2006; Anckaert et al., 2008; Kagan et al., 2008; Amor et al., 2009; Gjerris et al., 2009a, b), and median nuchal translucency (NT) MOM values have varied between no different to those in spontaneously conceived pregnancies to 1.6 MOM (Liao et al., 2001; Orlandi et al., 2002; Hui, et al., 2006a, b; Gjerris et al., 2009a, b). Hence, estimates of these markers tends to increase the rate of false positive trisomy results from the screening of IVF pregnancies (Heinonen et al., 1996; Orlandi et al., 2002; Tul, Novak-Antolic, 2006; Kagan et al., 2008; Amor et al., 2009; Gjerris et al., 2009a, b). The mechanisms behind these observed marker changes have been largely unexplored, but it has been speculated that the changes could be related either to fertility problems or to the procedures used in IVF (Heinonen et al., 1996; Liao et al., 2001; Tul and Novak-Antolic, 2006; Kagan et al., 2008). However, the effect of decreased fertility on DS screening markers has not been studied previously (Hui et al., 2006a, b; Gjerris et al., 2009a, b).

We anticipated that by examining the effects of subfertility alongside those of ART on first trimester DS screening, it would be possible to distinguish whether observed marker changes are due to decreased fertility or the IVF techniques used. For this purpose we analysed data from the Department of Gynaecology and Obstetrics, Kuopio University Hospital, and the Eastern Finland Laboratory Centre collected over a 3-year period between 2005 and 2007. The DS screening results of spontaneous pregnancies by TTP were compared with those of IVF pregnancies.

**Materials and Methods**

**Patients**

The study was retrospective and the data were collected from the Department of Gynaecology and Obstetrics of Kuopio University Hospital and the Eastern Finland Laboratory Centre. Only data from pregnant women with normal singleton pregnancies was included. The women entering the study lived in the Kuopio catchment area and gave birth at Kuopio University Hospital between January 2005 and December 2007. All those in the study had entered first trimester DS screening in the catchment area.

Information concerning the pregnancies was collected in maternity care units of Kuopio University Hospital district and at Kuopio University Hospital. The information included: the age of the mother, the method of conception (spontaneous or IVF), self-reported TTP classified as such pregnancies carry a higher risk of adverse outcome. The register of births included only pregnancies extending to at least the 22nd gestational week, therefore miscarriages and induced abortions were excluded. The reference group (N = 1164) consisted of the spontaneous pregnancies with the shortest TTP, 0–12 months. The study groups were women reporting 13–24 months TTP (N = 112), women reporting ≥25 months to pregnancy (N = 70) and the IVF pregnancy group (N = 39). The size of the total study population was 1385 pregnant women.

**Method of screening**

The information collected in the first trimester combined screening of DS consisted of the maternal age, concentrations of S-fβ-hCG and S-PAPP-A and the NT of the fetus. The screening was performed according to the recommendation of the Finnish Ministry of Social Welfare, and the markers were measured at their most ideal and representative point in time (Wald et al., 2003). The maternal serum samples were collected in maternity care units during Weeks 9 + 0 to 13 + 0. The NT and crown-rump length measurements were performed at health care centres and the Kuopio University Hospital maternity clinic by ultrasound-trained midwives and gynaecologists between Weeks 10 + 0 and 13 + 6. The serum samples were analysed in the Eastern Finland Laboratory Centre in Kuopio. The concentrations of S-fβ-hCG and S-PAPP-A were measured by time-resolved fluoroimmunoassays using an Auto-DELFIA kit (PerkinElmer Wallac, Turku, Finland). The risk figures for biochemical tests were calculated using LifeCycle software (PerkinElmer LifeSciences, Wallac, Turku, Finland). The risk figure programme compares a patient’s results with a population model described by a set of multivariate Gaussian distributions, taking into account the maternal age, crown-rump length, and fetal NT thickness. We used the cut-off limit of 1:250 for a fixed false-positive rate of 5%. Both the fβ-hCG and PAPP-A results were corrected for maternal weight and diabetes, although fβ-hCG measurements were only adjusted for smoking. The results were given as MOMs relative to values recorded for normal pregnancies at specific weeks. The within- and between-assay variation were both <3.4% in the detection range of 4–157 ng/ml for S-fβ-hCG and <2.4 and <4.0%, respectively, in the detection range between 44 and 7300 mU/l for PAPP-A. The analytical sensitivity for S-fβ-hCG and S-PAPP-A was 0.2 ng/ml and 5 mU/l, respectively. Quality assurance was performed under the supervision of an international quality assurance company (UK NEQAS, Edinburgh, Great Britain). In the present study, the effect of smoking was evaluated by comparing the screening results among smokers and non-smokers.

**Statistical analysis**

The parameters of the variables were tabulated and the differences between subjects and controls were tested for statistical significance. The MOMs of maternal serum concentrations of PAPP-A and fβ-hCG in study and control pregnancies were compared using a two-tailed pooled t-tests after log10 transformation of the maternal serum PAPP-A and fβ-hCG concentrations, expressed in MOMs. Logarithmic transformation of the data allowed us to use parametric tests, since the serum marker levels appeared to fit (log) Gaussian distributions. The medians of the marker levels and non-parametric tests are presented. Continuous variables such as the mean maternal age, birthweight and duration of the pregnancy were compared using Student’s two way t-tests, and χ2 tests were used to analyse dichotomous variables. Fisher’s exact was used in tests where there were fewer than five statistic units in any of the classes. P-values less than 0.05 were considered statistically significant. Data were analysed using the SAS software (SAS Institute Inc., Cary, NC, USA).
This study was approved by the Ethnical Research Committee of Kuopio University hospital and the Committee has given permission for the results to be published.

## Results

### The patient data

Table I shows the clinical characteristics of the mothers in terms of TTP and the method of conception. The mean maternal age increased with increasing TTP ($P < 0.01$). The proportion of overweight women ($P < 0.01$) and those with chronic diseases ($P = 0.04$) and prior miscarriages ($P < 0.01$) also increased significantly with increasing TTP. In comparison, the prevalence of these risk factors in the IVF group was closer to those with no delay in getting pregnant than those with prolonged TTP. The characteristics of the pregnancies are presented in Table II. The mean duration of pregnancy was similar in the groups with TTP of 0–12 and 13–24 months, but 3–4 days shorter in the longest TTP and IVF groups (although these differences were statistically insignificant). The mean birthweight was 256.4 g lower in IVF pregnancies than in the reference group ($P < 0.01$). The occurrence of pre-eclampsia was highest in the longest TTP group and lowest in the IVF group, but these differences were not statistically significant in comparison with the reference group. The proportion of infants that were SGA was highest in the IVF group, but the differences between them and infants in the other groups were again not statistically significant. The proportion of SGA in the study population was 9.4% and for all SGA-cases pooled together, the median PAPP-A among SGA pregnancies was 0.85 MOM whereas for pregnancies resulting in infants with normal weights PAPP-A was 0.98 MOM, the difference being statistically significant ($P < 0.01$). The occurrence of preterm delivery was significantly statistically higher in the IVF group than in the reference group ($P = 0.01$). There were no statistically significant gender differences between the groups.

### DS screening

The results of the first trimester DS screening are presented in Table III. The most significant finding was that the median and geometric mean MOM PAPP-A concentration was clearly lower in the

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**Table I Maternal characteristics of the study groups and the reference group**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>No infertility treatment</th>
<th>Assisted reproduction</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TTP 0–12 months (n = 1164)</td>
<td>TTP 12–24 months (n = 112)</td>
</tr>
<tr>
<td></td>
<td>%</td>
<td>%</td>
</tr>
<tr>
<td>Age ≥ 36 years</td>
<td>200</td>
<td>17.2</td>
</tr>
<tr>
<td>Pre-pregnancy, BMI ≥ 25</td>
<td>314*</td>
<td>28.6</td>
</tr>
<tr>
<td>Chronic illness (including hypertension and diabetes)</td>
<td>189</td>
<td>16.2</td>
</tr>
<tr>
<td>Smoking during pregnancy</td>
<td>101</td>
<td>8.7</td>
</tr>
<tr>
<td>Prior infertility treatment</td>
<td>11</td>
<td>0.9</td>
</tr>
<tr>
<td>Prior miscarriages</td>
<td>203</td>
<td>17.4</td>
</tr>
<tr>
<td>Prior induced abortions</td>
<td>25</td>
<td>2.1</td>
</tr>
<tr>
<td>Prior fetal death</td>
<td>6*</td>
<td>0.5</td>
</tr>
</tbody>
</table>

*Data missing with less than 6% of cases.

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**Table II Pregnancy characteristics and outcome in the study groups and the reference group**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>No infertility treatment</th>
<th>Assisted reproduction</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TTP 0–12 months (n = 1164)</td>
<td>TTP 13–24 months (n = 112)</td>
</tr>
<tr>
<td></td>
<td>%</td>
<td>%</td>
</tr>
<tr>
<td>Mean maternal age (years)</td>
<td>29.1</td>
<td>31.5</td>
</tr>
<tr>
<td>Mean birthweight (g)</td>
<td>3506.3</td>
<td>3436.5</td>
</tr>
<tr>
<td>Pre-eclampsia, %</td>
<td>61, 5.2%</td>
<td>7, 6.2%</td>
</tr>
<tr>
<td>Duration of the pregnancy (days)</td>
<td>277.6</td>
<td>277.1</td>
</tr>
<tr>
<td>Preterm birth (N%), %</td>
<td>65, 5.6%</td>
<td>6, 5.4%</td>
</tr>
<tr>
<td>Small for gestational age (N%), %</td>
<td>109, 9.4%</td>
<td>9, 8.0%</td>
</tr>
</tbody>
</table>

TTP, time-to-pregnancy.
group with TTP ≥ 25 months and the IVF group compared with the reference group, and these differences were statistically significant (P < 0.01 in both cases). However, the median and geometric mean MOM β-hCG concentration appeared to be statistically similar between the groups. The median and geometric mean MOM of NT was slightly, but not significantly, higher in IVF pregnancies than spontaneous pregnancies. A separate analysis was run to compare median PAPP-A MOMs among smoking and non-smoking women in each study group, but no significant differences were found between them.

The proportion of screening positives was between 2.1 and 2.7% in the reference group, the group with 13–24 months TTP, and the IVF group. The study group with the longest TTP (≥25 months), had the highest rate of positive screens (12.9%), and a significantly higher rate than the control group (P < 0.01). The false positive rate in this group was biased due to the higher age of the women, but the result remained statistically significant after adjusting for this factor.

### Discussion

The present study has demonstrated, for the first time, that subfertile women (defined as those with TTP of 2 years or more) had significantly reduced serum PAPP-A concentrations in comparison to those showing normal fertility, whereas their maternal serum β-hCG levels and NT measurements were similar to reference values. This screening profile was similar to that observed in IVF pregnancies. Along with higher maternal age, the lower concentrations of PAPP-A observed led to a higher false positive rate in DS screening in women with a prolonged TTP than in the reference group. Prolonged TTP has been considered a marker of subfertility, thus the main difference between IVF women and those with prolonged time to spontaneous pregnancy was the application of assisted reproduction techniques. Therefore, the results of the present study suggest that the mechanism behind alterations in the first trimester PAPP-A concentrations could be related to low fertility rather than to the use of ART.

Low PAPP-A has been reported to be associated with adverse pregnancy outcomes, such as preterm birth and LBW (Ong et al., 2000; Kwik and Morris, 2003; Tul et al., 2003; Liu et al., 2004; Smith et al., 2004; Barrett et al., 2008; Brameld et al., 2008; Spencer et al., 2008a, b). In the present study, however, the occurrence of pregnancy complications was similar between groups. In the group with the longest TTP, the mean maternal age was highest, there were more obese mothers, and the mothers had the highest probabilities of having had prior miscarriages or being treated for infertility prior to the index pregnancy. They also had the highest probability of presenting with a chronic disease. Obesity is known to be associated with reduced fecundity (Gesink and Maclehose, 2007; Yilmaz et al., 2009), and associations have been found between chronic diseases such as hypertension and increased risks of adverse pregnancy outcomes (Mavalankar et al., 1992; Catov et al., 2008). PAPP-A has been studied for diagnostic or predictive value in chronic diseases, where its concentration has been increased (Crea and Andreotti, 2005; Eleesber et al., 2006; Coskun et al., 2007; Liu et al., 2008) rather than decreased, and therefore the low PAPP-A in the longest TTP group can be assumed to be associated with subfertility, not with chronic diseases of the mother.

Previous studies have suggested that the risk of adverse obstetric outcomes, such as preterm birth and LBW, increase along with a longer TTP or a prolonged menstruation—conception interval (Joffe and Li, 1994; Gardosi and Francis, 2000). In addition, women with a long TTP may have tended to have had a LBW themselves (Nohr et al., 2009). In the present study, growth restriction from early pregnancy or under-estimation of true gestation might have explained the lower than normal PAPP-A results, but we did not find any discrepancies between dates in early ultrasound data or differences in birthweights corrected for gestational age between the groups. Furthermore, the concentrations of maternal serum β-hCG were comparable between the groups, suggesting that placental function...
was equal independently of the group studied. The novel finding of the present study was that the low PAPP-A concentration observed could be associated with decreased fertility rather than with fertility treatment, however, the underlying mechanisms behind subfertility remained speculative and largely unknown.

For PAPP-A, the risk calculation program for the first trimester DS screening does not take into account smoking or any chronic diseases recorded for the mother, other than insulin-treated diabetes. However, a separate analysis of the smokers in the study did not alter our conclusions. In the present study, the group with the longest TTP had the highest false positive rate in screening, raising the question of whether there is a need for PAPP-A adjustment in the risk calculation for the TTP. The weighting of PAPP-A in the calculation of DS risk is lower than that of NT but higher than that of β-hCG. However, the fact that the mean maternal age was significantly higher in women with prolonged TTP than in the reference group also needs to be considered. Therefore, the observed difference in PAPP-A concentration is likely to have contributed in concert with maternal age to the calculation of DS risk, and further studies are needed before considering adjustments in the risk calculations for TTP.

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