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# The impact of fetal gender on first trimester nuchal translucency and maternal serum free $\beta$-hCG and PAPP-A MoM in normal and trisomy 21 pregnancies

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**Objective** To investigate if fetal sex has an impact on 1st trimester combined screening for aenuploidy.

**Methods** We studied the first trimester PAPP-A, free $\beta$-human chorionic gonadatropin ($\beta$-hCG) and nuchal translucency levels in 56 024 normal, singleton pregnancies with known fetal sex at birth. We also examined the distributions in 722 pregnancies with trisomy 21 of known fetal sex.

**Results** We have found a 14.74% increase in first trimester maternal serum (MS) median free $\beta$-hCG MoM, 6.25% increase of PAPP-A and a 9.41% decrease in delta NT, when the fetus was female. Analysis of data has shown that women carrying a female fetus were 1.084 times more likely to be in the 'at risk' group than those carrying a male fetus. In examining data from 722 pregnancies in which the fetus was affected by trisomy 21, we observed a similar 20.8% increase in free $\beta$-hCG MoM, 5.7% increase in PAPP-A and a 12% decrease in delta NT when the fetus was female. Amongst the trisomy 21 cases, 88.8% of male trisomy 21 cases were detected compared with 91.2% in female cases, this difference was not statistically significant. Correcting for fetal sex redressed the balance in screen-positive rate between the sexes and had a minimal impact on detection rate.

**Conclusion** Correcting for fetal sex may be a worthwhile consideration. A cost-benefit analysis would be required to determine if it is feasible to introduce fetal gender assignment into the routine first trimester scan for the purpose of marker correction and whether this would have any significant impact. Copyright © 2009 John Wiley & Sons, Ltd.

KEY WORDS: fetal sex; prenatal screening; aneuploidy, Down's syndrome; free $\beta$-hCG; PAPP-A

## INTRODUCTION

The current optimum screening practice for the detection of fetal chromosomal anomalies is the first trimester measurement of maternal serum (MS) PAPP-A and free $\beta$-human chorionic gonadatropin ($\beta$-hCG) combined with fetal nuchal translucency (the combined test). With a fixed false-positive rate of 5%, this method detects around 90% of trisomies 21, 18 and 13, triploidy and sex chromosome aneuploidies (Spencer *et al*., 1999, 2000b; Tul *et al*., 1999).

Various factors such as maternal weight (Neveux *et al*., 1996; Spencer *et al*., 2003), ethnicity (Spencer *et al*., 2005) and smoking status (Spencer *et al*., 2004) are known to have an influence on MS marker levels, and these need to be corrected for in order to calculate an accurate patient-specific risk. Another potential parameter for correction currently under investigation is fetal gender. Several studies looking at second trimester pregnancies have suggested that fetal sex alters MS markers. Pregnancies with female fetuses have been observed to have significantly lower alpha fetal protein (MSAFP) (Calvas *et al*., 1990; Lockwood *et al*., 1993; Szabo *et al*., 1995; de Graaf *et al*., 2000) and higher free $\beta$-hCG (MS $\beta$-hCG) or total hCG levels than those of male fetuses (Brody and Carlstroem, 1965; Leporrier *et al*., 1992; Bazzett *et al*., 1998; Spencer, 2000). More recent studies have shown that, although levels of these markers in the second trimester are significantly different in karyotypically normal female fetuses, there is no significant differences between these markers in male and female Down syndrome cases (Ghidini *et al*., 1998; Spencer, 2000; Mueller *et al*., 2005); although Spong *et al*. found the converse (Spong *et al*., 1999). Further evaluation of the effect of fetal gender in prenatal screening has lead scientists to observe variances in first trimester MS markers. We have previously shown in a relatively small study ($n = 2923$) that fetal gender, determined at birth, may also have had an impact on the first trimester markers. In normal pregnancies, the presence of a female fetus caused 15 and 10% increases in MS free $\beta$-hCG and PAPP-A ($p < 0.00001$) and a 3% decrease in fetal NT ($p < 0.01$) (Spencer *et al*., 2000a).

In this study, we aim to establish these findings using a much larger cohort of normal pregnancies and those affected with Down syndrome, in order to discover how fetal gender may act upon detection rates of fetal aneuploidy in the first trimester of pregnancy, and to

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discuss the practicality of correcting for fetal sex if it were considered necessary.

## METHODS

Pregnancies for women attending routine first trimester maternity screening at King George Hospital, Essex; Queens Hospital, Essex; Harold Wood Hospital (now closed), Essex; Queen Elizabeth the Queen Mother Hospital, Kent; William Harvey Hospital, Kent; and Kent and Canterbury Hospital, Kent for births between June 1998 and July 2007 were examined.

Screening data were collected in the first trimester at the $10-13^{+6}$ weeks period and stored in a central database. MS free $\beta$-hCG and PAPP-A were measured using the Kryptor analyser (Brahms AG, Berlin) and the performance of this assay has been described previously (Spencer *et al*., 1999). NT was measured during an ultrasound examination by sonographers who had obtained the Fetal Medicine Foundation certificate of competence in the first trimester scan. Using patient specific gestational age, MS markers were converted to MoMs with correction where appropriate for smoking status, ethnicity and maternal weight. NT was converted to delta NT (Spencer *et al*., 2003), and patient specific risks were calculated using the maternal age at screening and the Fetal Medicine Foundation algorithm.

Birth data (including fetal sex) were collected at birth by the delivering hospital and stored in several individual databases, which were merged. A Structured Query Language (SQL) query was created to join screening and birth records. The selection criteria were: to match mothers, either (1) surname, first five letters of the forename (to allow for typographical errors) and DOB, or (2) surname and hospital number, or (3) first five letters of forename and hospital number (to allow for changing of surnames from marriage). Also, to match pregnancies, the date of conception derived from gestational age at screening and at birth had to be within 30 days to ensure the same pregnancy was selected for the individual woman match.

Software used: MySQL Community Server (version 5.0, MySQL AB, Uppsala, Sweden), SPSS (SPSS Inc., Chicago, Illinois).

To supplement the Down syndrome group additional information from cases screened at the Fetal Medicine Centre, London or at Harris Birthright Research Centre for Fetal Medicine were included.

An assessment of the impact of correcting for fetal sex, using the parameters derived from this study, was performed using the individual pregnancy data in this study.

## RESULTS

During the period of time studied, 56 334 normal, singleton pregnancies void of pregnancy complications, with full records for first trimester screening and birth data (including fetal sex) were retrieved. Of these, 28 726 (51.0%) were male and 27,608 (49.0%) were female. Table 1 shows the demographic data for the two fetal genders. Chi-square and Mann-Whitney tests showed no significant differences in the demographic data between the two groups.

From the Harold Wood screening laboratory, we identified 307 pregnancies with Down syndrome and from the Fetal Medicine Centre/Harris Birthright a further 416 cases were included. Of the 723 cases, 368 (50.9%) were male and 355 (49.1%) were female. Table 1 shows the demographic data for the two fetal genders. Again Chi-square and Mann-Whitney tests showed no significant differences in the demographic data between the two groups.

The median MoM free $\beta$-hCG and PAPP-A and delta NT values of the unaffected population are shown in table 2, along with the percentage change caused by the presence of a female fetus. Using the marker MoMs or the delta NT and the nonparametric Kruskal-Wallis test, all marker differences between the presence of male or female fetuses are significant ($p < 0.0001$).

Women had a high estimated risk of carrying a trisomy 21 fetus (1 in 300 or greater) in 3.22% of cases where the fetus was female and 2.97% where the fetus was male (Chi-square $p = 0.12$). Therefore, women carrying a female fetus were 1.08 times more likely to be in the 'at risk' group than those carrying a male fetus.

In the Down syndrome population the median MoM free $\beta$-hCG and PAPP-A and delta NT values are shown

Table 1—Demographic data in unaffected and Down pregnancies for each fetal gender population, median (range) or percentages

| | Unaffected | | Down | |
|---|---|---|---|---|
| | Male | Female | Male | Female |
| *n* | 28 726 | 27 608 | 368 | 355 |
| Maternal weight (kg) | 65.5 (29.6–187.0) | 65.5 (33.0–172.0) | 64.5 (35.4–171.2) | 65.0 (34.2–175.0) |
| Maternal age (years) | 29.3 (13.8–56.0) | 29.3 (13.3–52.5) | 37.68 (18–48) | 38.00 (16–49) |
| Gestational age (days) | 88 (77–97) | 88 (77–97) | 89 (70–97) | 89 (72–97) |
| Smokers (%) | 17.95 | 17.39 | 10.47 | 11.08 |
| Ethnic Origin (%) | | | | |
| Caucasian | 74.1 | 73.9 | 90.2 | 89.3 |
| Asian | 11.9 | 12.0 | 2.0 | 4.9 |
| Afro-carribean | 8.2 | 8.1 | 4.2 | 2.9 |
| Oriental | 1.1 | 1.1 | 1.1 | 0.6 |
| Other | 4.7 | 4.8 | 2.5 | 2.6 |



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Table 2—Median MoM free $\beta$-hCG and PAPP-A and delta NT values for male and female fetuses and the percentage (%) difference between male and female fetuses in the unaffected and Down population

| | Unaffected | | | | | Down | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Male | Female | All | % Change | $p$–Kruskal-Wallis | Male | Female | All | % Change | $p$–Kruskal-Wallis |
| FB MoM | 0.95 | 1.09 | 1.01 | 14.74 | <0.0001 | 1.870 | 2.251 | 2.000 | 20.3 | <0.0001 |
| PAPP-A MoM | 0.96 | 1.02 | 0.99 | 6.25 | <0.0001 | 0.530 | 0.560 | 0.549 | 5.7 | 0.484 |
| Delta NT | −0.0172 | −0.0597 | −0.0397 | −9.41 | <0.0001 | 1.514 | 1.378 | 1.401 | −9.0 | 0.517 |

in Table 2, along with the percentage change caused by the presence of a female fetus. Using the marker MoMs or the delta NT and the non parametric Kruskal-Wallis test, only the difference in free $\beta$-hCG between the presence of male or female fetuses was significant ($p < 0.0001$) although the higher PAPP-A MoM and the lower delta NT in the females was in the same direction of travel as found in the unaffected population.

When we examined the number of cases detected, we observed a detection rate of 88.8% in the presence of a male fetus and 91.2% in the presence of a female fetus. Using the Chi- square test this difference did not reach statistical significance with a Pearson Chi-square statistic of 1.14 and $p = 0.2856$.

Using the unaffected and affected study population data we made adjustments to the delta NT and median MoM's based on the known fetal sex and the factors in Table 2 for unaffected pregnancies. For example, if the fetal sex was female we divided the observed free $\beta$-hCG MoM by 1.09, the observed PAPP-A MoM by 1.02 and the delta NT was corrected by −0.0597. We then re-calculated the patient specific risk and calculated the screen-positive rate at a 1 : 300 risk cut off. We found that using this procedure the screen-positive rate in the presence of a female fetus was 3.12%, almost identical to that in the presence of a male fetus (3.10%). In the affected population the detection rates were also equivalent—being 89.7% in the presence of a male and 90.2% in the presence of a female

## DISCUSSION

The results of this study have shown significant fetal sex differences in MS free $\beta$-hCG and PAPP-A, and in delta NT for both normal pregnancies and for MS free $\beta$-hCG amongst pregnancies with trisomy 21. We have found a 14.74% increase in first trimester MS median free $\beta$-hCG MoM when the fetus being carried was female. In the second trimester, free $\beta$-hCG values have also been found to be higher in normal female pregnancies in both amniotic fluid (Spencer *et al*., 1997) and MS (Spencer, 2000).

In this study we have also found that the presence of a female fetus causes a 6.25% increase in MS median PAPP-A MoM, and a 9.41% decrease in delta NT. The findings in this study support a smaller study ($n = 2923$), where we have previously shown in normal female pregnancies a 15% increase in median MoM free $\beta$-hCG (Spencer *et al*., 2000a), as well as being substantiated in other studies (Yaron *et al*., 2002; Ardawi *et al*., 2007). Previously we found the increase in PAPP-A levels to be 10% levels and NT to be decreased by 3–4%. (Spencer *et al*., 2000a).

Overall the changes in marker levels contribute to a small increase in false-positive rate and a small increase in detection rate in cases with trisomy 21 amongst women carrying a female fetus. Correcting for fetal sex was shown to redress the imbalance in the detection rates and false-positive rates between the presence of a male or female fetus. Whether this change is significant enough to warrant correction needs wide discussion. The size of the change in the level of free $\beta$-hCG amongst women with a female fetus is similar to the reduction in PAPP-A amongst smokers—where it is considered almost mandatory to correct for—a logical argument, therefore, could be proposed that correcting for fetal sex should be undertaken.

Fetal sex assignment by ultrasound in the first trimester is becoming more efficient and accurate. Using the 'sagittal sign' (Emerson *et al*., 1989; Mazza *et al*., 2001; Efrat *et al*., 2006). Mazza *et al*. (2001) were able to identify the fetal gender with 100% accuracy, from as early as $12^{+0}$ weeks. Female fetuses were correctly assigned (100%) from $11^{+2}$ to $11^{+6}$ weeks, while at this particular time of gestation only 46% of males were identified. Similar results were presented from Efrat *et al*. (2006), where the accuracy of the assignment of the studied pregnancies has been shown to be correct in 99–100% of the male at all stages of gestation studied, and from 91.5% of the female group at $12–12^{+3}$ weeks to 100% at $13–13^{+6}$ weeks. A different approach for fetal gender determination is the use of maternal peripheral blood. Since Lo *et al*. (1998) first described the presence of free fetal DNA in the maternal circulation, several groups have shown that using techniques such as real-time PCR for particular genes (SRY, DYS14), fetal gender is diagnosed with 100% sensitivity and specificity from the first trimester of pregnancy (Costa *et al*., 2001; Sekizawa *et al*., 2001; Honda *et al*., 2002; Hyett *et al*., 2005). A combination of both methods (fetal DNA and ultrasound) has been suggested for monitoring sex linked disorders (Chi *et al*., 2006).

A cost benefit analysis would need to be carried out to determine whether the introduction of fetal assignment in the first trimester would be feasible for the predicted balancing of detection rate and false-positive rate. If fetal sex determination is introduced in first trimester screening, it will be necessary to consider the creation of new policies and legal frameworks, to protect the rights of unborn children and prevent sex selection for



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non-medical reasons (Hall *et al*., 2006) as happens in India (George, 2006) and China (Lai-wan *et al*., 2006).

In conclusion, according to results presented in this large-scale study, fetal gender appears to have an effect on first trimester serum markers, $\beta$-hCG and PAPPA, and the sonographic parameter, delta NT. This fact causes variations in the calculated risk for trisomy 21 and results in clinically differentiated false-positive rates and detection rates.

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