First-Trimester Screening for Down Syndrome in Singleton Pregnancies Achieved by Intrauterine Insemination

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Purpose: To evaluate whether achieving a singleton pregnancy by IUI affects the results of first-trimester screening for Down syndrome compared to naturally conceived pregnancy.

Methods: Forty-nine IUI and 3059 naturally conceived singleton pregnancies were included in the study. Ovulation in IUI pregnancies was induced by clomiphene and human menopausal gonadotropin. Progesterone was given after insemination for 2 weeks. Down syndrome screening was performed using a combination of maternal age, fetal nuchal translucency, and maternal serum concentrations of free β -hCG and PAPP-A during the period of 10–14 weeks' gestation.

Results: In IUI pregnancies, nuchal translucency thickness and the levels of PAPP-A were significantly higher. The values of free β -hCG were not statistically different between the two groups. The screen-positive rate in IUI pregnancies was significantly higher (14.3% vs. 7.1%).

Conclusions: Singleton pregnancies achieved by IUI have a higher screen-positive rate. Not only elder maternal age but also exogenous hormones given during the process of ovulation induction and after conception may play an important factor influencing positive screening results.

KEY WORDS: Down syndrome; intrauterine insemination; ovulation induction; screening.

INTRODUCTION

Screening for Down syndrome (DS) in the first trimester can be achieved by consideration of a combination of variables including maternal age, fetal nuchal translucency (NT) thickness, and levels of maternal serum free β -human chorionic gonadotropin (β -hCG) and pregnancy-associated plasma protein-A (PAPP-A). This combined ultrasonographic and biochemical-screening strategy has been reported to achieve a detection rate of 85% with a 5% false-positive rate (1–5). There is some evidence that second-trimester screening for DS is associated with

a higher false-positive rate in pregnancies achieved by assisted reproduction than in naturally conceived pregnancies, because of an unexplained increase in maternal serum hCG concentration and a decrease in unconjugated estriol (6–11). To the best of our knowledge, there has been limited study of the effect of achieving a pregnancy by intrauterine insemination (IUI) on the results of screening for DS in the first trimester. This study evaluated whether achieving a singleton pregnancy by IUI affects the results of combined first-trimester screening for DS compared to natural conception.

MATERIALS AND METHODS

Patient Selection

From April 1999 to January 2002, 3059 patients with spontaneously conceived singleton pregnancies and 49 singleton pregnancies achieved by IUI, who

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received combined test for DS screening (maternal serum free β -hCG, PAPP-A, and fetal NT measurement) at 10–14 weeks' gestation at Cathay General Hospital were included in this retrospective study. Demographic data, ultrasound findings, and the results of biochemical testing were retrieved from our computerized obstetrical records.

Ovarian Stimulation and Intrauterine Insemination

Ovulation stimulation was started on Day 3 to Day 5 of the menstrual cycle with the regimen of clomiphene citrate 100 mg per day and human menopausal gonadotropin (hMG) 150 IU per day. Ovarian response was monitored with transvaginal ultrasound and the daily dose of hMG was adjusted accordingly. Ovulation was induced with 10,000 IU hCG when at least two follicles measuring more than 18 mm in diameter were observed. The prepared semen was transferred into the uterine cavity 48 h after hCG injection.

Luteal Phase Support

Natural micronized progesterone 300 mg per day was given orally in three divided doses for 2 weeks after insemination.

Screening for Down Syndrome

First trimester screening for DS was performed at 10–14 weeks' gestation using a combination of maternal age, fetal NT thickness, and maternal serum concentrations of free β -hCG and PAPP-A. Three well-trained sonographers measured fetal NT thickness with an ultrasonography unit (Acuson 128, Mountain View, CA). Fetal NT measurement was based on the criteria published by the Fetal Medicine Foundation in the UK (12). Three NT scans were made in each case, and the largest value was chosen for calculating DS risk. Gestational age was determined by fetal crown-rump length (CRL) at the time of NT measurement. Only fetuses with CRL between 36 and 76 mm were included in the analysis. This corresponds to a gestational age of between 10 and 14 weeks.

Blood samples were collected at the time of fetal NT scan, and the serum was separated into aliquots and stored at -20°C on the same day. Maternal serum concentrations of free β -hCG and PAPP-A were determined within 1 week of collection by micro titer plate enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's protocol (Genemed Biotechnologies, USA). Fetal NT, maternal serum

free β -hCG, and PAPP-A values were divided by the day-specific median to determine the multiple of median (MoM) for each marker. DS risk was calculated using Alpha software (Logical Medical Systems, UK) based on a multivariate Gaussian distribution as described by Wald and Hackshaw (13). Values of 1 in 400 or greater were considered to indicate high risk for DS.

Statistical Analysis

Statistical analysis of data was performed with SPSS 8.0 software (Smart Software, Leeds). All of the free β -hCG, PAPP-A, and NT values were compared between the two groups using Student's *t*-test. A *p*-value of <0.05 was considered statistically significant.

RESULTS

The basic clinical data and serum screening results of both groups are shown in Table I. There were no significant differences between the two groups in fetal CRL and gestational age determined at the time of screening. Maternal age ($p < 0.05$) and body weight ($p = 0.033$) in the IUI group were significantly higher than in the control group.

The NT measurements in the IUI group (median = $1.20 \pm 0.61\text{MoM}$) were significantly greater than those of the control group (median = $1.03 \pm 0.58\text{MoM}$) ($p = 0.034$). The levels of PAPP-A were significantly higher in the IUI group (median = $1.18 \pm 0.77\text{MoM}$) than in the control group (median = $0.99 \pm 0.63\text{MoM}$) ($p = 0.038$). Although the values of free β -hCG of the IUI group (median = $1.23 \pm 0.86\text{MoM}$) were slightly

Table I. Comparison of First-Trimester Screening Markers in Intrauterine Insemination Versus Naturally Conceived Singleton Pregnancies

	Controls	IUI	<i>p</i> value
No. of patients	3059	49	
Maternal age (years)	29.0 ± 3.2	31.1 ± 2.8	$p < 0.05$
Maternal weight (kg)	54.0 ± 7.7	56.4 ± 7.0	$p < 0.05$
CRL (mm)	41.2 ± 25.2	41.3 ± 22.1	ns
Gestation (days)	83.9 ± 5.9	82.3 ± 4.3	ns
Median NT(mm)	1.00 ± 0.52	1.07 ± 0.49	ns
Median NT in MoM	1.03 ± 0.58	1.20 ± 0.61	$p < 0.05$
Median free β -hCG in MoM	1.14 ± 0.76	1.23 ± 0.86	ns
Median PAPP-A in MoM	0.99 ± 0.63	1.18 ± 0.77	$p < 0.05$

Note. Data are presented as Mean $\pm$ SD. *p* value is calculated with Student's *t* test. ns – Not significantly different.

higher than those of the control group (median = $1.14 \pm 0.76\text{MoM}$), this difference was not significant ($p = 0.409$) (Fig. 1(A)–(C)).

Seven patients (14.3%) with IUI achieved pregnancies had a positive screening result. Two of them had undergone amniocentesis which revealed trisomy 21 (47, XY, +21) in one. Increased NT thickness (MoM = 3.14), higher free β -hCG (MoM = 1.11), and lower PAPP-A (MoM = 0.63) was found in the affected case. In the control group, 216 cases (7.1%) had a positive-screening result for DS.

DISCUSSION

Our study confirms previously reported observations that assisted reproduction increases the screening-positive rate in first-trimester screening for DS (14). In this study, 14.3% of the women who achieved pregnancy with IUI had a positive-screening result, which was significantly higher than in the naturally conceived pregnancy group (7.1%). The higher screen-positive rate in the IUI group might have been secondary to older maternal age, higher

Fig. 1. Box plots show the MoM values of screening markers between the control and IUI group. The horizontal line in the center of the box shows the median value, the upper and lower limits of the box show the interquartile range, and the whiskers represent 5 and 95% quartiles for each group. Asterisks represent extremes and circles represent outliers. **(A)**, The β -hCG distribution of the control (1.14 ± 0.76) and IUI (1.23 ± 0.86) groups. **(B)**, The NT distribution of the control (1.03 ± 0.58) and IUI (1.20 ± 0.61) groups. $p < 0.05$. **(C)**, The PAPP-A distribution of the control (0.99 ± 0.63) and IUI (1.18 ± 0.77) groups. $p < 0.05$.

Fig. 1. (Continued).

maternal weight, and other factors. It is tempting to relate the increased frequency of positive screening for DS in IUI achieved pregnancy to extraplacental products coming from the greater numbers of corpora lutea, undiagnosed multiple early implantations, unknown metabolic disturbances, as well as exogenous hormones given during the process of ovulation induction (15). Therefore, accurate risk estimates in first-trimester screening for DS in pregnancy achieved following controlled ovarian stimulation requires correction of the measured concentrations of serum markers to reduce the overestimation of risk. However, before such adjustment of risk calculation is considered, it would be necessary to study a large number of DS pregnancies achieved by assisted reproduction techniques involving ovarian stimulation to deter-

mine the effectiveness of screening with adjusted means.

According to previous studies, the false-positive rates of serological markers increase with maternal age in second-trimester screening for Down syndrome (16). The fact seem exist in first-trimester screening (2–4). We analyze 3059 cases in the control group in this study. The false-positive rate of screening is 4.5% between 20 and 25; 5.4% between 25 and 30; 8.9% between 30 and 35 years of age, respectively. It seems that age plays an important variable affect the false-positive rate in first-trimester screening for Down syndrome. In this study, the mean age in the IUI group is older than that in the control group (31.1 ± 2.8 vs. 29.0 ± 3.2). If we adjust the control group equal in age to the IUI group, the expectant false-positive rate is 8.9% in the IUI group as equal as that in the control group. However, the false-positive rate we observed is higher than expected (14.1% vs. 8.9%). It suggests that more confounding factors than elder age result in higher false-positive rate in the IUI group.

Previous reports on first-trimester screening in singleton pregnancies achieved by assisted reproduction found that neither fetal NT, free β -hCG nor PAPP-A were significantly different from their values in spontaneously conceived pregnancies (14,17). In this study, the IUI group had significantly higher levels of PAPP-A and increased NT thickness compared with the control group. There were also slightly increased levels of free β -hCG in the IUI group compared to the control group. Our results suggest that controlled ovarian stimulation before conception and progesterone support after conception may play an important factor influencing positive DS screening results. Therefore, the marker changes induced by this treatment would be more significant in the first trimester. Further studies will be needed to explore the mechanism in the future.

Liao *et al.* (14) and Wojdemann *et al.* (17) reported that concentrations of maternal serum free β -hCG in IVF pregnancies were significantly higher than those in naturally conceived pregnancies in the first trimester (14,17). This increase is similar to those reported in the second trimester both for free β -hCG and for total hCG (8–11). In this study, we found slightly increased levels of free β -hCG in the IUI group but no significant difference in this value between the two groups. Most protocols of ovarian stimulation in IVF are intended to produce more mature follicles, whereas IUI stimulates less ovulation of oocytes (18). The lack of significant free β -hCG elevation in this study may reflect that the degree of

ovarian stimulation may be one of the confounding factors, which affects the concentration of maternal serum free β -hCG in the first trimester.

CONCLUSION

The findings of this study suggest that the first-trimester screening positive rate for DS in singleton pregnancies achieved by IUI is higher than that for natural conceptions. In addition to elder maternal age, gonadotropin given during the process of ovulation induction and progesterone given for luteal phase support after conception may play an important factor influencing positive DS screening results. The mechanism is underdetermined.

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