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Screening for Down syndrome in dichorionic twin pregnancies conceived by in vitro fertilization (IVF): a clinical pilot study to confirm the laboratory methods

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Abstract
Purpose The corrections necessary to estimate the risk for Down syndrome in twin pregnancies have been pointed out. We performed a nested controlled study to evaluate the validity of these corrections in dichorionic twins conceived by IVF.
Methods Detailed clinical data was collected from the medical records. Twins were matched with a contemporaneous cohort of spontaneously conceived singleton pregnancies that serve as reference in a 1 to 4 ratio. All patients had their entire obstetrical care at our Hospital. The Student t-test was used for group comparisons and a p-value <0.05 was considered significant.
Results Nineteen sets of normal twins concordant in size and with appropriate weight for gestational age were matched with 80 normal and mature newborns. Significant differences between groups were found for maternal age, gestational age at delivery and newborn weight (all $p<0.01$). No statistical differences were noted for the levels of the biochemical markers expressed as multiples of the median. However, a 15 % closer approximation to the laboratory median for PAPP-A and a 10 % closer approximation to the laboratory median for free β-hCG was evident in twins when compared to the reference group.
Conclusions These findings support the methods used to estimate the risk for Down syndrome in dichorionic twin pregnancies conceived after IVF. A future study with a larger sample size could confirm if the laboratory corrections done on the combined screening test improve the predictability of Down syndrome in dichorionic twin pregnancy conceived by IVF when compared to singleton pregnancies.

Keywords Twin pregnancies · IVF · First trimester combined screening test · Down syndrome screening · Free β-hCG · PAPP-A

Capsule For women with Dichorionic twins after IVF the combined first trimester test is an appropriate Down syndrome screening test.

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Introduction

Twins account for approximately 3.3 % of all births In the USA [1]. Early sonographic evaluations in twin pregnancies allows for accurate estimation of the expected due day, chorionicity and placenta location/s [2]. Ultrasound imaging also allows for assessment of the relationships between the amniotic sac/s and the cervix and to rule out major fetal structural abnormalities [3–5]. Compared to singleton gestations, twins also have an increased risk for chromosomal abnormalities [6, 7]. For these reasons, prenatal screening for common genetic syndromes should be offered to all women expecting twins. Indeed, in twin pregnancies the risk for Down syndrome (DS) can be calculated using the 11–14 week combined screening test where corrections for maternal weight, ethnicity, smoking, in-vitro fertilization (IVF), chorionicity, and age of the egg-donor are applied [8–10]. In this test, the likelihood of affected versus unaffected is

estimated for each twin and then combined and divided by 2 to produce the "pseudo risk" for the pregnancy. Therefore, contrary to singleton pregnancies, the "pseudo risk" reported in twin pregnancies is theoretical and not based on data collected from large cohorts of DS pregnancies [11]. At the moment, scarce evidence supporting the validity of the recommendations that have been made for the first trimester combined screening test in DC twins conceived after IVF is available. For these reason we designed this nested-controlled clinical study to validate the methods used by commercial laboratories to estimate the risk for Down syndrome in dichorionic twin pregnancies conceived after IVF. The null hypothesis was that the levels of free β-hCG and PAPP-A reported per fetus after the first trimester combined DS screening tests would be similar between normal twin pregnancies conceived after IVF and spontaneously conceived normal singleton pregnancies.

Material and methods

The study was performed at a University based Reproductive Genetics Unit between May 2010 and April 2012 and was approved by the Hospital institutional review board. Clinical variables from all pregnancies were obtained from the medical records and recorded on an electronic database. Patients with DC twin pregnancies conceived by IVF that delivered during the third trimester of pregnancy normal newborns with appropriate weight for gestational age were included in the study. The reference was composed of contemporaneous normal mature singletons with appropriate weight for gestational age. All patients had an estimated DS risk of less than 1 in 250 after the 11–14 week combined DS screening test. NT measurements were obtained by certified sonographers. Patients that underwent antenatal interventions that could affect the maternal serum-free β-hCG and PAPP-A values were excluded.

Statistical analysis Each twin pregnancy was matched in a 1 to 4 ratio with singleton pregnancies to allow for a greater precision of the probability estimate. We calculated a sample size that would achieve 80 % power for detecting at least a 25 % difference in maternal serum-free β-hCG and PAPP-A for the laboratory multiples of median MoM at a probability level of 0.05. A two-tailed Student t-test for independent samples was used for comparisons between groups and a p-value <0.05 was considered significant.

Results

Two-hundred and twenty five patients with multiple pregnancies had prenatal evaluations at our center during the study period. Of these, 19 had triplets, 101 delivered at a different hospital, three resulted in non-viable pregnancies, 17 were monochorionic-diamniotic twins and 64 did not have a combined first trimester screening test. Of the remaining, two conceived without assisted reproductive technologies leaving 19 phenotypically normal sets of DC twins conceived after IVF without a history of CVS or amniocentesis, cerclage, stillborn, congenital anomalies, or a newborn with a birthweight above the 90th or below the 10th percentile for gestational age. All twins were born after 30 weeks gestation and were concordant in size. The 5 min Apgar score was greater than eight and all twins were evaluated by a neonatologist. These 19 sets of twins were matched with 80 healthy newborns which were product of singleton pregnancies that were spontaneously conceived and born at term with normal birthweights.

Significant differences between groups were noted for maternal age, gestational age at delivery and newborn weight (all $p<0.01$). No statistical differences were noted for the maternal serum-free β-hCG and PAPP-A MoM. Table 1 summarizes the results and shows a 15 % closer approximation to the laboratory median for PAPP-A and a 10 % better approximation to the laboratory median for free β-hCG in twins when compared to the reference group.

Discussion

The principal finding from this controlled pilot study in which pathology that could affect the results was excluded, is that the corrected maternal serum concentrations of free β-hCG and PAPP-A from dichorionic twin pregnancies conceived after IVF adjust to the reference. This agrees with the predictions made by other authors using different methodologies [8–11]. Our secondary finding is that sonographic imaging together with the corrected maternal serum markers might improve the efficiency of the combined 11–14 week DS screening test in dichorionic twin pregnancies conceived after IVF when compared to the reference group. This observation would be relevant for genetic counseling and for discussing alternative management plans with patient's carrying twins after IVF if they are confirmed by an independent study with a larger sample size.

The combined 10–14 week screening test can be used in twin gestations [6–13]. This test combines maternal age, the sonographic measurements of the fetal nuchal translucency for the embryonic crown-rump length and the maternal serum concentration of PAPP-A and free-beta-hCG expressed in MoM for gestational age. In dichorionic twins (DC), the presence of an affected twin may be masked by an unaffected twin and for that reason the estimated detection rates for the combined 10–14 week screening test varies from 84 % in

Table 1 Down syndrome screening markers in normal dichorionic twin pregnancies conceived after IVF and a singleton reference

	Mat age years	CRL mm	Free β-hCG MOM per fetus	PAPP-A MOM per fetus	NT mm Twin A	NT mm Twin B	GA Delivery weeks	NB-WT gm	NBWT %Diff
Singleton $N=80$	32 (5.4)	60 (7.1)	1.20 (0.6)	1.3 (0.8)	1.5 (0.4)	Same	39.4 (1.0)	3441 (407)	NA
Twins $N=19$	35 (4.4)	62.4 (10)	1.05 (0.5)	1.2 (0.6)	1.4 (0.3)	1.6 (0.4)	34.6 (3.3)	2200 (600)	12.5 %
p-value	0.008	0.13	0.15	0.21	0.27	0.15	<0.001	<0.001	NA

Summary statistics expressed as mean and standard deviations mean (SD). The two-tailed Student t-test for independent samples was used to make comparisons between groups and a p-*value* <0.05 was considered significant. Compared to singleton pregnancies twins had a 15 % lower PAPP-and a 10 % lower free β-hCG. The overall maternal serum Median values of 2.1 MoM for free β-hCG and 2.4 MoM for PAPP-A are within the ranges previously reported for chromosomally normal twin pregnancies [10]

DC dichorionic, *Mat* maternal, *CRL* fetal crow-rump length, *free β-hCG* free beta human chorionic gonadotropin, *MOM* multiples of the median, *PAPP-A* pregnancy associated plasma protein-A, *NT* nuchal translucency, *GA* gestational age, *NBWT* newborn weight, *Diff* difference

monochorionic twins (MC),to 70 % in DC, and 72 % for all twins for a fixed 5 % false positive rate [11]. Presently in the United States the estimated frequency of DS after 20 weeks gestation is 1 in 2000, most likely as a reflection of the implementation of antenatal screening tests [14].

This study was not geared towards the investigation of the antenatal detection rate of DS in DC twin pregnancies conceived by IVF. The study was design to validate the methods used in the combined 11–14 week test to produce the DS risk estimates in DC twins conceived after IVF. Indeed, we calculated the deviations from the laboratory median for free β-hCG and PAPP-A using information extracted from the medical records. This analysis performed in normal twin pregnancies conceived after IVF and a contemporary group of normal singleton pregnancies offered an indirect metric of the efficiency of the DS risk estimate in dichorionic twins conceived after IVF. The results confirmed the null hypothesis and agree with the prediction that the corrected maternal serum concentrations of free β-hCG and PAPP-A from the group of twins adjusts to the reference. In addition, the analysis demonstrated a 15 % closer approximation to the laboratory median for maternal serum-PAPP-A and a 10 % closer approximation for the maternal serum-free β-hCG in twins than in singleton pregnancies. Together these findings provide a new argument in favor of using the combined 11–14 week DS screening test in patients with DC twins conceived after IVF [6–13, 15–17]. A new study with a larger sample size will be necessary to confirm that the laboratory corrections performed in the combined screening test improves the predictability of DS in dichorionic twin pregnancy conceived by IVF when compared to singleton control.

Strengths and limitations The major strength of this study is the simplicity of the design. Data from two distinct, homogeneous and contemporaneous groups of patients with normal newborns, a negative combined DS screening test and without antenatal interventions that could affect the interpretation of the results was compared. The maternal serum-free β-hCG and PAPP-A concentrations found are within the ranges that have been previously reported strengthening the validity to the observations (7). The study, however, also has limitations. First, the study was powered to detect at least a 25 % difference for the MoMs and we only found a 10–15 % difference between groups: based on our findings 130 twins and 400 singleton pregnancies are required to prove that a 10 % difference in MoMs is statistically significant. If a study with this sample size is performed and the difference is confirmed, the observation will be clinically meaningful given that about 120,000 sets of twins are delivered yearly just in the United States. The second limitation is that a karyotype analysis in the newborns was not performed. This could raise the question that some of the newborns were not normal. However, this uncertainty is minimal for three main reasons: 1) all patients had a negative antenatal DS screening test, normal antenatal sonographic imaging evaluations and the normal post natal evaluations by a neonatologists; 2) Rutgers-Robert Wood Johnson Medical School is a referral center for complicated pregnancies with experienced specialists in maternal fetal medicine, neonatology and clinical genetics and no clinical concerns regarding DS were reported on the medical records; 3) No anomalies were documented during the newborn hospital stay or by the parents after discharge from the Hospital.

In conclusion, we designed a pilot study that allowed for a head-to-head clinical comparison of the normal levels of maternal serum-free β-hCG and PAPP-A from dichorionic twin pregnancies conceived after IVF and a reference group. Our findings support the corrections that are made for the calculation of DS risk in dichorionic twin pregnancies conceived after IVF. Future studies could confirm if the predictability of Down syndrome in dichorionic twin pregnancy conceived by IVF is better when compared to singleton pregnancies.

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