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Medically assisted reproduction and second-trimester maternal serum marker screening for Down syndrome

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Objectives To evaluate the effect of *in vitro* fertilization (IVF) and intracytoplasmic sperm injection (ICSI) on total hCG, free ß-hCG, AFP and unconjugated estriol (uE3) used as markers for second-trimester Down syndrome maternal serum screening.

Methods Second-trimester maternal sera from 1515 singleton pregnancies (970 by IVF, 545 by ICSI) were compared with control sera (21 014 cases). Free ß-hCG, total hCG, AFP and uE3 were compared between the control group and the medically assisted reproduction groups. The percentages of at-risk patients ($\geq$1/250) were also compared.

Results No differences in values of the maternal serum markers were observed between the medically assisted and control groups. When maternal age was taken into account, the screen-positive rate for Down syndrome screening did not differ between the two groups.

Conclusion Patients undergoing assisted reproduction techniques can be counseled for maternal serum Down syndrome screening with the same efficacy as patients with naturally conceived pregnancies.

KEY WORDS: IVF; ICSI; medically assisted reproduction; trisomy 21; amniocentesis; maternal serum markers; Down syndrome screening

INTRODUCTION

Maternal serum biochemical markers for Down syndrome screening are known to be associated with various maternal factors. Some factors such as maternal weight affect the distribution of maternal markers (Cuckle, 1995), others affect the initial risk of Down syndrome due to maternal age, for example, a previous Down syndrome-affected child (Arbuzova *et al*., 2001), and others such as multiple pregnancy affect both marker distribution and maternal age risk (Spencer *et al*., 1994; Neveux *et al*., 1996; Meyers *et al*., 1997; Muller *et al*., 2003). As part of the screening process, it is important to know whether assisted reproduction techniques have any effect on the distribution of biochemical markers that could result in a difference in screen-positive and/or detection rates. In recent years, several publications (Ribbert *et al*., 1996; Heinonen *et al*., 1996; Frishman *et al*., 1997; Lam *et al*., 1999; Barkai *et al*., 1996; Wald *et al*., 1999; Maymon and Shulman, 2001; Maymon and Shulman, 2002) have indicated that second-trimester maternal serum marker levels are altered in different forms of assisted conception pregnancies, mainly IVF (*in vitro* fertilization) with or without ICSI (intracytoplasmic sperm injection). We therefore undertook a study of a large number of patients treated by medically assisted reproduction techniques.

MATERIAL AND METHODS

Data for this retrospective study were provided by 15 of the 72 ABA laboratories authorized to carry out maternal serum marker screening in France. A total of 1515 singleton pregnancies in which assisted reproduction pregnancy was involved were identified during the period 1996–2002. Cases in which embryo reduction was performed were excluded. In the 1515 cases of medically assisted reproduction, IVF was performed in 970 cases, and IVF plus ICSI in 545 cases.

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Information collected for each pregnancy included maternal age, gestational age at maternal serum sampling, serum marker profile in multiples of the gestation-specific normal median (MoM), estimated Down syndrome risk. Although, the date of embryo transfer was known, the gestational age was estimated by first-trimester ultrasonography in all cases. Maternal serum screening was based on a two-marker test in 1385 (91.4%) cases: alpha-fetoprotein (AFP) and free ß-human chorionic gonadotrophin (hCG) in 522 (Perkin-Elmer Life Sciences, Turku, Finland–dual kit); AFP and total hCG in 863 (Abbott–Axsym; Perkin-Elmer; Bayer–ACS180). A three-marker test was performed in 130 cases (8.6%): uE_3, AFP and free ß-hCG (Perkin-Elmer). Each laboratory used its own normal medians, maternal weight correction equations and distribution parameters to calculate multiple of median (MoM). Estimated Down syndrome risk at the second trimester was recalculated in all cases with the same software (MultiCalc Perkin-Elmer) based on maternal age and two markers AFP and hCG or free ß-hCG. A 1/250 cut-off was adopted to define the screen-positive group.

The control population ($n = 21\,014$) consisted of three groups defined by the markers tested: 13 332 patients screened by the double test AFP and total hCG (Abbott; Roche), 6634 by the double test AFP and free ß-hCG (Perkin-Elmer), and 1048 by the triple-test AFP, total hCG and unconjugated estriol (Ortho Clinical Diagnosis).

Log-transformed MoMs were used for comparisons. Means for each marker were compared in medically assisted reproduction patients and controls using Student's t-test. In the case of significance, analysis of variance was completed by multiple comparison tests using the contrast method. Between-group comparison of the calculated risk adjusted for maternal age was performed using the Cochran-Mantel-Haenszel Chi square test. A p value <0.05 was considered statistically significant. Statistical analyses were performed using SAS v 8.2 software (SAS Institute Inc. USA).

RESULTS

Maternal age was statistically different in the medically assisted reproduction groups and the control group (Table 1).

The distribution of each marker in the medically assisted reproduction and control groups was analyzed and found to follow a normal law.

Medians, 5th and 95th percentiles of the four markers and for the different groups are presented in Table 2. No significant differences between the medically assisted reproduction groups and the control population were observed for any of the four marker values.

The number of patients included in the at-risk Down syndrome group (calculated risk $\geq$1/250) differed significantly between the two groups ($p < 0.0001$), but this difference disappeared when maternal age was taken into account (Table 3).

Table 1—Maternal age of control and of medically assisted reproduction patients

	Maternal age (years)	
	Median (25th–75th percentile)	Mean (±SD)
Control patients ($n = 21\,014$)	30 (27–33)	29.9 (±4.9)
Medically assisted reproduction ($n = 1515$)	33 (30–35)	32.5 (±3.8)**
IVF ($n = 970$)	33 (30–36)	32.7 (±4.1)**
IVF + ICSI ($n = 545$)	32 (30–35)	32.2 (±3.7)**

Comparison with control patients.
** $p < 0.0001$.

Table 2—Maternal serum marker values expressed in multiple of median (MoM) in patients with medically assisted reproduction

	AFP MoM	hCG MoM	free ß-hCG MoM	uE3 MoM
Control patients				
n	21 014	13 332	6634	1048
Median (5th–95th percentile)	1.03 (0.61–1.90)	1.03 (0.38–2.32)	1.02 (0.41–3.02)	1.02 (0.63–1.58)
Medically assisted reproduction				
n	1515	863	652	130
Median (5th–95th percentile)	0.96 (0.55–1.92)	1.06 (0.40–2.65)	1.08 (0.40–3.25)	0.95 (0.56–1.54)
IVF				
n	970	480	490	88
Median (5th–95th percentile)	0.97 (0.55–2.06)	1.10 (0.41–2.74)	1.05 (0.39–3.36)	0.90 (0.55–1.42)
IVF + ICSI				
n	545	383	162	42
Median (5th–95th percentile)	0.95 (0.53–1.78)	1.01 (0.38–2.40)	1.11 (0.39–3.18)	1.0 (0.56–1.90)

IVF, *in vitro* fertilization; ICSI, intracytoplasmic sperm injection.

Table 3—Percentage of patients with calculated risk of trisomy 21 ≥ 1/250

	MAR patients ($n = 1515$)		Control patients ($n = 21\,014$)	
	Percentage	At risk ≥ 1/250	Percentage	At risk ≥ 1/250
Total	100%	12.7%	100%	8.8%
<30 years	22.5%	3.1%	47.2%	3.0%
30–34 years	45.8%	9.1%	34.3%	7.9%
35–37 years	22.2%	18.7%	12.4%	17.7%
≥38 years	9.5%	39.3%	6.1%	40.2%

MAR = medically assisted reproduction.

Table 4—AFP and hCG MoM in assisted reproduction as reported in the literature

	AFP		hCG		Free-ßhCG		uE3	
Studies	IVF (n)	Controls	IVF	Controls	IVF	Controls	IVF	Controls
Ribbert *et al*., 1996	0.89 (67)	1.00	1.28 (67)	1.00				
Barkai *et al*., 1996	0.98 (327)	1.00	0.93 (298)	1.00			0.92 (261)	1.00
Heinonen *et al*., 1996	1.02 (41)	1.00	1.52 (41)	1.00				
Frishman *et al*., 1997	0.95 (69)	1.03	1.22 (69)	0.99			0.90 (69)	0.98
Lam *et al*., 1999	0.88 (42)	0.94	1.15 (42)	0.94				
Wald *et al*., 1999	0.99 (151)	1.00	1.14 (151)	1.00	1.09 (151)	1.00	0.94 (151)	1.00
Maymon and Shulman, 2001	1.04 (46)	1.00	1.38 (46)	0.99			1.11 (46)	1.01
Bar-Hava *et al*., 2001	1.13 (70)	1.02	1.31 (70)	0.95			0.98 (70)	1.01
Perheentupa *et al*., 2002	0.98 (96)	—	1.20 (96)	—				
Raty *et al*., 2002	0.95 (58)	1.00			1.19 (58)	1.00		
Overall	0.98 (967)	1.00	1.21 (939)	0.99	1.14 (209)	1.00	0.93 (597)	1.00
Present study	0.97 (970)	1.03	1.10 (480)	1.03	1.05 (490)	1.02	0.90 (88)	1.02

DISCUSSION

In this study, the differences observed (6% lower in AFP, 7% higher in hCG, 12% lower in uE3) between the medically assisted reproduction and control groups in the four markers of second-trimester Down syndrome maternal serum screening were not statistically significant. However, they tended to be in the same direction as in many previously published studies (see Table 4). The number of IVF patients included in these studies (41 to 69) was probably too small, because in one study with more patients (Barkai *et al*., 1996) median values of AFP and hCG were not statistically different from controls, as in the present study.

The same discrepancies were noted in the number of at-risk patients. Frishman *et al*. (1997) and Wald *et al*. (1999) observed a higher percentage than in control groups (30.4% vs. 14.4% and 27.8% vs. 16.6%, respectively) in age-matched comparisons, and Heinonen *et al*. (1996) reported percentages of 26.8% versus 6.6% in a nonage-matched population. The small study populations are sufficient to explain these differences and when a standardized maternal age distribution was used, Wojdemann *et al*. (2001) observed no difference (4.7% vs. 4.9%). The values observed for IVF plus ICSI are not discussed here due to the small (23 to 48) study populations (Lam *et al*., 1999; Perheentupa *et al*., 2002; Raty *et al*., 2002).

The same discrepancies between studies were observed during the first trimester, as Orlandi *et al*. (2002) observed no difference in free ß-hCG and a 21% difference in PAPP-A, and Wojdemann *et al*. (2001) observed no difference with the same markers. Studies based on first-trimester nuchal translucency measurement also presented discrepancies, probably because of small study populations (Maymon *et al*., 1999; Orlandi *et al*., 2002).

In conclusion, second-trimester Down syndrome maternal serum screening can be used in medically assisted reproduction patients (IVF or IVF plus ICSI) with the same efficiency as in controls.

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REFERENCES

Arbuzova S, Cuckle H, Mueller R, Sehmi I. 2001. Familial Down syndrome: evidence supporting cytoplasmic inheritance. *Clin Genet* **60**: 456–462.

Bar-Hava I, Ytzhak M, Krissi H, *et al*. 2001. Triple-test screening in *in vitro* fertilization pregnancies. *J Assist Reprod Genet* **18**: 226–229.

Barkai G, Goldman B, Ries L, Chaki R, Dor J, Cuckle H. 1996. Down's syndrome screening marker levels following assisted reproduction. *Prenat Diagn* **16**: 1111–1114.

Cuckle H. 1995. Improved parameters for the risk estimation in Down's syndrome screening. *Prenat Diagn* **15**: 1057–1065.

Frishman GN, Canick JA, Hogan JW, Hackett RJ, Kellner LH, Saller Jr DN. 1997. Serum triple-marker screening in *in vitro* fertilization and naturally conceived pregnancies. *Obstet Gynecol* **90**: 98–101.

Heinonen S, Ryynanen M, Kirkinen P, Hippelainen M, Saarikoski S. 1996. Effect of *in vitro* fertilization on human chorionic gonadotropin serum concentrations and Down's syndrome screening. *Fertil Steril* **66**: 398–403.

Lam YH, Yeung WS, Tang MH, Ng EH, So WW, Ho PC. 1999. Maternal serum alpha-fetoprotein and human chorionic gonadotropin in pregnancies conceived after intracytoplasmic sperm injection and conventional in vitro fertilization. *Hum Reprod* **14**: 2120–2123.

Maymon R, Dreazen E, Rozinsky S, Bukovsky I, Weinraub Z, Herman A. 1999. Comparison of nuchal translucency measurement and midgestation serum screening in assisted reproduction versus naturally conceived singleton pregnancies. *Prenat Diagn* **19**: 1007–1011.

Maymon R, Shulman A. 2001. Comparison of triple serum screening and pregnancy outcome in oocyte donation versus IVF pregnancies. *Hum Reprod* **16**: 691–695.

Maymon R, Shulman A. 2002. Serial first- and second-trimester Down's syndrome screening tests among IVF-versus naturally conceived singletons. *Hum Reprod* **17**: 1081–1085.

Meyers C, Adam R, Dungan J, Prenger V. 1997. Aneuploidy in twin gestations: When is maternal age advanced? *Obstet Gynaecol* **89**: 248–251.

Muller F, Dreux S, Dupoizat H, *et al*. 2003. Second-trimester Down syndrome maternal serum screening in twin pregnancies: impact of chorionicity. *Prenat Diagn* **23**: 331–335.

Neveux LM, Palomäki GE, Knight GJ, Haddow JE. 1996. Multiple marker screening for Down syndrome in twin pregnancies. *Prenat Diagn* **16**: 29–34.

Orlandi F, Rossi C, Allegra A, *et al*. 2002. First-trimester screening with free ß-hCG, PAPP-A and nuchal translucency in pregnancies conceived with assisted reproduction. *Prenat Diagn* **22**: 718–721.

Perheentupa A, Ruokonen A, Tuomivaara L, Ryynanen M, Martikainen H. 2002. Maternal serum beta-HCG and alpha-fetoprotein concentrations in singleton pregnancies following assisted reproduction. *Hum Reprod* **17**: 794–797.

Raty R, Virtanen A, Koskinen P, *et al*. 2002. Serum free beta-HCG and alpha-fetoprotein levels in IVF, ICSI and frozen embryo transfer pregnancies in maternal mid-trimester serum screening for Down's syndrome. *Hum Reprod* **17**: 481–484.

Ribbert LS, Kornmann LH, De Wolf BT, *et al*. 1996. Maternal serum screening for fetal Down syndrome in IVF pregnancies. *Prenat Diagn* **16**: 35–38.

Spencer K, Salonen R, Muller F. 1994. Down's syndrome screening in multiple pregnancies using alpha-fetoprotein and free beta-hCG. *Prenat Diagn* **14**: 537–542.

Wald NJ, White N, Morris JK, Huttly WJ, Canick JA. 1999. Serum markers for Down's syndrome in women who have had *in vitro* fertilisation: implications for antenatal screening. *Br J Obstet Gynaecol* **106**: 1304–1306.

Wojdemann KR, Larsen SO, Shalmi A, Sundberg, Christiansen M, Tabor A. 2001. First-trimester screening for Down syndrome and assisted reproduction: no basis for concern. *Prenat Diagn* **21**: 563–565.

