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Maternal serum HCG is higher in the presence of a female fetus as early as week 3 post-fertilization

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BACKGROUND: Maternal serum HCG (MSHCG) is higher when the fetus is a female than when it is male. This has been demonstrated in the second and third trimesters of pregnancy, and recently at 10–14 weeks gestation. In this study we assessed whether this gender-related difference can be detected as early as week 3 post-fertilization. METHODS: The IVF setting was chosen because it provides precise dating of gestational age and early sonography for the number of gestational sacs. The study included 347 IVF cycles from 335 patients. Only pregnancies with a single implanted embryo that resulted in a single live birth of known gender were included. MSHCG was measured on days 14–20 post-fertilization, and levels were expressed as gestational age-corrected multiples of the median (MoMs). The $\log_{10}$ MSHCG MoMs were compared according to fetal gender. RESULTS: MSHCG levels were significantly higher (18.5%) in week 3 post-fertilization in the presence of a female fetus ($P < 0.0002$). CONCLUSION: Because a fetal gender-related difference in MSHCG can be demonstrated as early as week 3 post-fertilization, the difference may be attributed to placental factors and not to the effects of the fetal hypothalamic–hypophyseal–gonadal axis.

Key words: fetal gender/IVF/maternal serum HCG

Introduction

Fetal gender has been shown to have a significant influence on maternal serum levels of HCG (MSHCG). It was initially demonstrated that third trimester MSHCG is higher in women carrying a female fetus than in those with a male (Brody and Carlstrom, 1965; Hobson and Wide, 1974; Wide and Hobson, 1974; Danzer *et al.*, 1980; Deville *et al.*, 1980; Obiekwe and Chard, 1982). With the widespread utilization of second trimester biochemical screening for Down's syndrome, the same gender-related difference was also demonstrated in the second trimester in most studies (Leporrier *et al.*, 1992; Lockwood *et al.*, 1993; Santolaya-Forgas *et al.*, 1997; Bazzett *et al.*, 1998; Ghidini *et al.*, 1998; Spong *et al.*, 1999; Steier *et al.*, 1999; Spencer, 2000). Two recent studies found that maternal serum free β-HCG is also significantly higher in the late first trimester (10–14 weeks gestation) in women carrying female fetuses (de Graaf *et al.*, 2000; Yaron *et al.*, 2001).

The reason for the gender-related difference in maternal serum HCG has remained elusive since Brody and Carstrom first described this phenomenon in 1965 (Brody and Carlstrom, 1965). It has been suggested that the gender-related differences in MSHCG result from differential activity of the fetal hypothalamic–hypophyseal–gonadal axis (Obiekwe and Chard, 1982), thereby influencing fetal levels of pregnandiol (Rawlings and Krieger, 1964), progesterone, androgens (Boroditsky *et al.*, 1975), testosterone or estradiol (Danzer *et al.*, 1980), which in turn affect HCG production or utilization. Alternatively, it has been proposed that the gender-related difference in MSHCG is mediated by the sex chromosomes of the trophoblast, whereby some genes on the X chromosome that escape inactivation may be over-expressed by the placenta in the presence of a female fetus (Obiekwe and Chard, 1982, 1983).

Our hypothesis was that if the gender-related differences in MSHCG can be demonstrated prior to the establishment of the fetal hypothalamic–hypophyseal–gonadal axis, they may then be attributed to differential expression of genes by the trophoblast. We thus chose to determine whether the gender-related difference in MSHCG can be detected as early as week 3 post-fertilization, when MSHCG is usually first measured. Although maternal serum markers may be somewhat altered with IVF (Lam *et al.*, 1999), we chose this setting because the precise gestational age is documented to the day, multiple MSHCG measurements are available, and the number of gestational sacs is assessed sonographically at an early stage.

Materials and methods

Patients

The study included 335 patients who underwent 347 IVF cycles at the Racine IVF Unit, Tel Aviv Medical Center. Ovulation induction

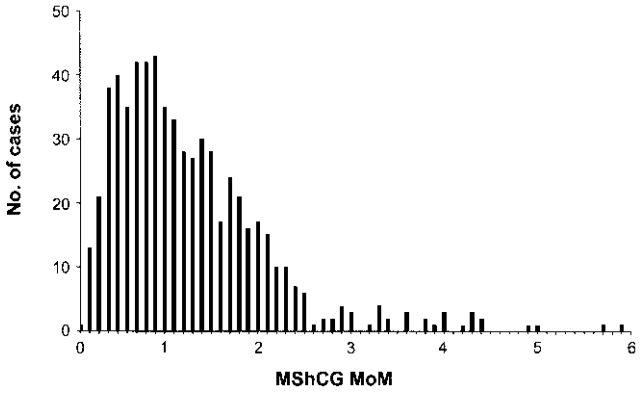

Figure 1. The distribution of patients according to gestational age-corrected multiples of the medians (MoMs) HCG. The distribution is compatible with a $\log_{10}$ (log-Gaussian) distribution.

was achieved by a standard protocol of GnRH analogue and HMG (Pergonal; Teva Pharmaceutical Industries, Israel) as described previously (Ben-Yosef *et al.*, 1999). Oocyte retrieval was scheduled 34–35 h after the administration of 10 000 IU HCG (Chorigon; Teva Pharmaceutical Industries, Israel). Insemination of the retrieved oocytes was performed by standard insemination or ICSI, according to sperm parameters. Embryo cleavage rate and quality were evaluated on day 2 or 3 and the best quality embryos were chosen for transfer. The number of transferred embryos varied according to patient age, the number of previously failed IVF cycles and embryo quality.

Maternal serum HCG and sonographic measurements

Maternal serum HCG was measured for each patient on 1–3 occasions, beginning on day 14 through day 20 post-fertilization. For measurement of MSHCG the IMMULITE® immunoassay was used (Diagnostic Product Corporation, Los Angeles, CA, USA). This assay has a coefficient of variation of 4.5–4.8% over the range of 103–3120 mIU/ml for HCG. The presence and number of gestational sacs was determined by transvaginal sonography, beginning 4–5 weeks post-fertilization.

Statistical analysis

Patient variables and pregnancy outcomes were retrieved from our in-house customized 'Computerized Fertilization' database. Data were categorized by gestational age in days and by fetal gender. Because MSHCG levels exhibit an initial rapid increase, expected levels for each day of gestation are significantly different. Thus, to allow comparison across various gestational ages, levels of MSHCG were expressed as gestational age-corrected multiples of the (daily) medians (MoMs), in a manner similar to biochemical screening in the first and second trimesters (Wald *et al.*, 1988). The advantage of using the median, rather than the mean, as a measure of the central tendency is that it is not influenced by occasional outlying values. Median MSHCG values were calculated for each day post-fertilization for male and female fetuses. For the purpose of statistical analysis, MoMs for the entire study group were calculated according to medians derived only from women in the study who carried a male fetus.

The distribution of MSHCG MoMs is skewed, as is shown in Figure I. However, $\log_{10}$ MSHCG MoM is distributed in a Gaussian manner over the whole range of values (Wald *et al.*, 1988). Therefore, the $\log_{10}$ MSHCG MoMs may be compared using a two-tailed non-paired Student's *t*-test, assuming equal variance, as previously described (Spencer, 2000).

To compare patient demographic and treatment variables, Student's *t*-test and χ^2 tests were used, as appropriate. A *P*-value < 0.05 was considered statistically significant.

Results

Of the 347 IVF pregnancies included in the study, 184 had a female fetus and 163 had a male fetus. Patients had 1–3 measurements of MSHCG, for a total of 642 values. When comparing patients carrying females with those having males, no statistically significant differences were noted for any of the patient demographic or treatment variables, including maternal age, number of previous pregnancies, number of previously failed IVF cycles, duration of infertility or indication for IVF treatment (Table I). Likewise, no differences were noted in the number of oocytes retrieved or the number of embryos transferred. As expected, the birthweight of male newborns was significantly higher than that of females (3172 versus 2972 g respectively, $P = 0.001$).

Table II shows the median MSHCG by gestational age in days, as classified by the presence of a female or male fetus, taking into account all measurements available for each patient. Median MSHCG levels are increased in the presence of a female fetus on day 16–20 post-fertilization. Table III shows the median MSHCG MoM, mean $\log_{10}$ MSHCG MoM, $\log_{10}$ SD and statistical significance, as classified by the presence of a female or male fetus on days 14–20 post-fertilization. MoMs were calculated according to medians derived only from women bearing males. When the $\log_{10}$ MSHCG MoMs were compared using Student's *t*-test, assuming equal variance, significantly higher levels were observed when the fetus was a female ($P = 0.00017$). Finally, when these comparisons were repeated, taking into account only a single measurement for each patient, the differences were still statistically significant ($P = 0.0038$)

Discussion

Despite numerous reports confirming the gender effect on MSHCG levels in the second and third trimesters of pregnancy, and in the late first trimester (10–14 weeks gestation) (de Graaf *et al.*, 2000; Yaron *et al.*, 2001), no satisfactory explanation for this phenomenon has been provided. Brody and Carlstrom first demonstrated that maternal serum total HCG levels in the third trimester were significantly elevated in women with a female fetus compared with those with a male (Brody and Carlstrom, 1965). They suggested that this may be the result of pregnandiol excretion in the latter half of pregnancy, basing their hypothesis on observations made by Rawlings and Krieger, who noted a high male to female ratio in fetuses of mothers with a high pregnandiol excretion (Rawlings and Krieger, 1964). Several subsequent reports confirmed this observation, but only in the third trimester.

Crosignani *et al*. also noted significantly higher amniotic fluid HCG concentrations when the fetus was female than when it was male (Crosignani *et al.*, 1972). Hobson and Wide reported that the concentration of HCG in the placenta was lower when the fetus was male (Hobson and Wide, 1974; Wide and Hobson, 1974), and suggested that this gender-related

Table I. Patient demographic and treatment variables classified by the presence of a female or male fetus

Variable	Males (n = 163)	Females (n = 184)	P-value
Maternal age (years)	32.7 ± 5.0	32.4 ± 4.6	NS
Previous pregnancies	1.16 ± 1.17	1.12 ± 1.42	NS
Previous IVF cycles	4.4 ± 3.7	4.4 ± 3.4	NS
Duration of infertility (years)	5.4 ± 3.8	5.0 ± 3.8	NS
Indication for IVF			
Mechanical	52	51	
Male	51	75	
Unexplained	39	36	
Others	21	22	
Total	163	184	NS
No. of oocytes retrieved	12 ± 7	10 ± 7	NS
No. of embryos transferred	3 ± 1	3 ± 1	NS
Birthweight (g)	3172 ± 599	2972 ± 538	0.001

Table II. Daily median maternal serum HCG (MSHCG) in week 3 post-fertilization, classified by the presence of a female or male fetus

Days post-fertilization	Males		Females	
	No. of measurements	Median MSHCG (mIU/ml)	No. of measurements	Median MSHCG (mIU/ml)
14	10	70	10	61
15	40	98.5	38	96.5
16	50	111	71	132
17	69	200	70	230
18	73	255	67	345
19	43	401	41	517
20	32	518	28	678
Total	317	–	325	–

Table III. Comparison of MSHCG on days 14–20 post-fertilization between patients carrying females and those with males

	Males (n = 317)	Females (n = 325)	P value[a]
Median MSHCG MoM	1.000	1.185	
Mean $\log_{10}$ MSHCG MoM	–0.0193	0.0612	0.00017
SD $\log_{10}$ MSHCG MoM	0.3010	0.2932	

[a]Two-tailed non-paired Student's t-test

difference may be due to a differential rate of inactivation or utilization of HCG by the fetus or the mother, or that the male and female gonads differentially regulate placental gonadotrophin production. Boroditsky *et al*. suggested that the difference in MSHCG between female- and male-bearers may indicate that the fetus exerts control over placental HCG production, but acknowledged that a primary genetic difference in the placental function cannot be ruled out (Boroditsky *et al.*, 1975). They further speculated that higher levels of progesterone present in the umbilical arteries of males may have an inhibitory effect on HCG production, resulting in lower HCG levels in male placentas. They also suggested that androgens originating from the male fetus may suppress HCG production by the placenta.

Danzer *et al*. evaluated the feasibility of using third trimester MSHCG levels for predicting fetal sex, but found that this was impractical (Danzer *et al.*, 1980). They suggested that the fetal steroid milieu may, in part, regulate placental production of HCG. As support for this hypothesis they pointed out a significant inverse correlation between cord-blood testosterone and estradiol levels of male infants and MSHCG. In a series of 96 normal pregnancies, Deville *et al*. noted that MSHCG, its a subunit and particularly its β-subunit were higher in cases of female fetuses, although this did not reach statistical significance (Deville *et al.*, 1980).

Obiekwe and Chard confirmed previous observations that MSHCG is higher in women carrying female fetuses only in late pregnancy (Obiekwe and Chard, 1982). They proposed that the most obvious explanation for this phenomenon would be some specific relation to the development of the fetal pituitary–gonadal system. They also theorized that the difference may be 'mediated... at a more fundamental level, by the sex chromosomes of the trophoblast.' Indeed, in a subsequent article, Obiekwe and Chard evaluated the maternal serum levels of other placental proteins as well, including human placental lactogen (HPL), Schwangerschaft protein (SP_1) and placental protein 5 (PP_5). They found that HPL, as well as HCG, is increased in mothers carrying female fetuses, but found no change in SP_1 or PP_5 (Obiekwe and Chard, 1983).

They concluded that the earlier lines of speculation, attributing the sex difference in MSHCG to the potential role of HCG as a gonadotrophin in the fetus may be excluded, since 'This argument cannot be easily stretched to include HPL or placental steroid sulfatase.' They suggested that, 'The synthesis of placental proteins might be related to the number of X chromosomes, with almost complete inactivation in some cases (SP_1, PP_5), partial inactivation in others (HCG, HPL) and no inactivation with steroid sulfatase (STS).' While this theory certainly applies to STS which is mapped to chromosome X and is known to escape inactivation (Migeon *et al.*, 1982), neither the genes for HCG nor for its receptor are located on the X chromosome.

With the advent of biochemical screening for Down's syndrome in the second trimester, several studies found significantly higher levels of MSHCG in the presence of a female fetus (Leporrier *et al.*, 1992; Lockwood *et al.*, 1993; Santolaya-Forgas *et al.*, 1997; Bazzett *et al.*, 1998; Ghidini *et al.*, 1998; Spong *et al.*, 1999; Steier *et al.*, 1999; Spencer, 2000). This was contrary to earlier studies (reviewed above) and even some recent reports (Steier *et al.*, 1999), which failed to demonstrate any significant gender-related difference in MSHCG in the second trimester. The apparent discrepancy may be partly explained by the relatively large size of the study populations in later studies and to the statistical analysis using multiples of the medians (MoMs) and $\log_{10}$ transformations, which are more appropriate for such comparisons. Leporrier *et al*. analysed MSHCG in 3000 patients at 16–20 weeks of gestation and noted that MSHCG was significantly higher in the presence of a female fetus after 17 weeks gestation (Leporrier *et al.*, 1992). They suggested that the underlying mechanism may be due to the high levels of testosterone observed in male fetuses just before mid-gestation (Reyes *et al.*, 1974). In a series of over 10 000 patients, we have demonstrated that patients with female fetuses had significantly higher MSHCG and lower α-fetoprotein at 14–22 weeks gestation (Bazzett *et al.*, 1998).

The fetal gender-associated differences in MSHCG may potentially result in a higher computed Down's syndrome risks in patients with female fetuses, who are more likely to have 'screen positive' results than patients with male fetuses (Spong *et al.*, 1999). However, Spencer suggested that despite the significant fetal gender-related differences in marker levels, there is no evidence to suggest that this results in any significant gender bias in Down's syndrome detection rates by maternal serum screening in the second trimester (Spencer, 2000).

First trimester combined screening for Down's syndrome now uses sonographically determined nuchal translucency, maternal serum free β-HCG and pregnancy-associated plasma protein-A (PAPP-A) (Wald and Hackshaw, 1997). de Graaf *et al*. noted a significant increase in free β-HCG in female fetuses (de Graaf *et al.*, 2000). We have recently also demonstrated that the median free β-HCG MoM at 10–13 weeks gestation is significantly higher (19%) in the presence of a female fetus (Yaron *et al.*, 2001).

The results of the present study confirm that MSHCG is significantly increased in the presence of a female fetus as early as day 16 post-fertilization. Taken together, the data presented in this, and previous studies, suggest that MSHCG is consistently elevated in the presence of a female fetus, throughout gestation. The fact that this phenomenon occurs as early as week 3 of embryonic development cannot be explained by the role of the fetal hypothalamic–hypophyseal–gonadal axis as previously suggested, because at this stage the necessary organs have yet to develop (Moore and Presaud, 1998). The gender-related differences should therefore be attributed to differential expression of placental proteins by female compared with male fetuses. This would be in agreement with the hypothesis put forth by Obiekwe and Chard regarding the possible escape from inactivation of some X-linked genes that play a role in the metabolism of HCG in the placenta (Obiekwe and Chard, 1982, 1983). It would be tempting to speculate that genes in the X chromosome pseudoautosomal regions escape inactivation and are therefore over-expressed in females. However, the genes for β-HCG and its receptor are located on chromosomes 19q13.32 and 2p21 respectively. The explanation may lie elsewhere in the complex mechanisms that regulate HCG production by the early trophoblast (Sorensen *et al.*, 1995). Two of the factors known to influence HCG production by the placenta are GnRH (Khodr and Siler-Khodr, 1978; Islami *et al.*, 2001) and γ-aminobutyric acid (GABA), via GABA-A-like receptors (Licht *et al.*, 1992). The genes for at least two subunits of the GABA-A receptor [α3 and ε (OMIM #305660 and #300093 respectively)] have been mapped to chromosome Xq28 near the second pseudoautosomal region where some genes escape X-inactivation. These genes, and possibly others, would be attractive candidates for molecular analysis of the differential expression of genes by the female and male placentas.

In conclusion, the fact that a gender-related difference in MSHCG exists as early as week 3 post-fertilization suggests that there is a differential expression of genes by the placentas of female compared with male fetuses. While the gender-related difference in MSHCG is statistically significant, it has little value in predicting fetal sex because of the small proportion of pregnant women with serum HCG concentrations that are high or low enough to allow a prediction with high probability (Danzer *et al.*, 1980). A model for predicting fetal sex may be generated if additional first trimester markers also demonstrate such a gender-related difference. This concept is biologically plausible, since gender-related differences have been described for other second trimester maternal serum markers; α-fetoprotein is lower (Sowers *et al.*, 1983; Petrikovsky, 1989; Calvas *et al.*, 1990; Bazzett *et al.*, 1998) and inhibin-A is higher (Lam and Tang, 2001) in the presence of a female fetus.

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